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(54) Title: OX-2 COSTIMULATORY MOLECULE

(57) Abstract

Methods and compositions for using the OX-2 protein to modulate a T cell mediated immune response are described. Novel structure forms of the OX-2 T cell costimulatory molecules also are described. These structural forms comprise a novel structure domain or have a structural domain deleted. The structural forms correspond to naturally-occurring alternatively-spliced forms of OX-2 T cell c stimulat ry molecules or variants thereof which can be produced by standard recombinant DNA techniques. The novel structure forms of the OX-2 T cell costimulatory molecules can be used to identify agents which stimulate the expression of alternative forms of costimulatory molecules and to identify components of the signal transduction pathway which results in costimulation of T cells.

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OX-2 COSTIMULATORY MOLECULE

Government Support

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Related Applications

This invention claims priority from a provisional application, US Serial No.60/008,754, filed December 8, 1995.

Field of the Invention

This invention relates to methods and compositions for modulating a T cell mediated immune response in a subject. The methods and compositions involve administering to the subject the OX-2 protein or a T cell costimulatory molecule related to the OX-2 protein.

Background of the Invention

For CD4+ T lymphocyte activation to occur, two distinct signals must be delivered by antigen presenting cells to resting T lymphocytes (Schwartz, R.H. (1990) Science 248: 1349-1356; Williams, I.R. and Unanue, E.R. (1991) J Immunol. 147:3752-3760; Mueller, D.L. et al., (1989) J Immunol. 142:2617-2628). The first, or primary, activation signal is mediated physiologically by the interaction of the T cell receptor/CD3 complex (TcR/CD3) with MHC class II-associated antigenic peptide and gives specificity to the immune response. The second signal, the costimulatory signal, regulates the T cell proliferative response and induction of effector functions. Costimulatory signals are pivotal in determining the functional outcome of T cell activation since delivery of an antigen-specific signal to a T cell in the absence of a costimulatory signal results in functional inactivation of mature T cells, leading to a state of tolerance (Schwartz, R.H. (1990) Science 248:1349-1356).

Molecules present on the surface of antigen presenting cells which are involved in T cell costimulation have been identified. These T cell costimulatory molecules include murine B7-1 (mB7-1; Freeman, G.J. et al., (1991) J Exp. Med. 174:625-631), and the more recently identified murine B7-2 (mB7-2; Freeman, G.J. et al., (1993) J Exp. Med. 178:2185-2192). Human counterparts to the murine B7-1 and B7-2 molecules have also been described (human B7-1 (hB7-1) Freedman, A.S. et al., (1987) J Immunol. 137:3260-3267; Freeman, G.J. et al., (1989) J Immunol. 143:2714-2722; and human B7-2 (hB7-2); Freeman, G.J. et al., (1993) Science

262:909-911; Azuma, M. et al. (1993) Nature 366:76-79). Novel forms of B7-1 and B7-2 are described in international application PCT/US95/02576, which claims priority to U.S. Serial No. 08/205,697. The B7-1 and B7-2 genes are members of the immunoglobulin gene superfamily; the B7-1 and B7-2 proteins each contain a two immunoglobulin domain extracellular structure comprised of IgV-like and IgC-like domains.

Both B7-1 and B7-2 are counter-receptors for two ligands, CD28 and CTLA4, expressed on T lymphocytes (Linsley, P.S. et al., (1990) Proc. Natl. Acad Sci. USA 87:5031-5035; Linsley, P.S. et al., (1991) J Exp. Med. 174:561-569). CD28 is constitutively expressed on T cells and, after ligation by a costimulatory molecule, induces IL-2 secretion and T cell proliferation (June, C.H. et al. (1990) Immunol. Today 11:211-216). CTLA4 is homologous to CD28 (Linsley, P.S., Protein Science 3:1341-1343 (1994)) and appears on T cells after activation (Freeman, G.J. et al. (1992) J Immunol. 149:3795-3801). Although CTLA4 has a significantly higher affinity for B7-1 than does CD28, its role in T cell activation appear to be that of negative regulation (Tivol et al. (1995) Immunology, 3:541-547) remains to be determined. It has been shown that antigen presentation to T cells in the absence of the B7-1/CD28 costimulatory signal results in T cell anergy in vitro (Gimmi, C.D. et al. (1993) Proc. Natl. Acad. Sci. USA 90:6586-6590; Boussiotis, V.A. et al. (1993) J Exp. Med. 178:1753). The ability of T cell costimulatory molecules such as B7-1 and B7-2 to bind to CD28 and/or CTLA4 on T cells and trigger a costimulatory signal in the T cells provides a functional role for these molecules in T cell activation.

Like B7-1 and B7-2, OX-2 is a member of the immunoglobulin gene superfamily; the OX-2 protein contains a two domain extracellular structure comprised of IgV-like and IgC-like domains. The rat OX-2 (alternatively referred to herein as rOX-2) protein was first defined using antibodies developed in mice against Type Ia glycoproteins purified from rat thymocytes by lectin chromatography (Barclay et al., Immunogenetics 25(2):133-135 (1987)). The rat OX-2 protein is expressed on a variety of cells, including, for example, thymocytes, neurons, endothelium, B cells and follicular dendritic cells. The rat OX-2 cDNA was cloned by Clark et al., EMBO 4(1):113-118, 1985 and shown to encode a type I transmembrane protein with a leader, an extracellular IgV-like and IgC-like domain and a transmembrane/cytoplasmic domain. The inclusion of OX-2 into the immunoglobulin supergene family and the relationship of the OX-2 primary structure to the T cell receptor (TCR)-beta chain and Ig light chains also have been reported. The human OX-2 homologue (hOX-2) was cloned by McCaughan et al., Immunogenetics 25:329-335 (1987) and shown to be encoded by at least four exons spread over

>10 kb. Cell hybrid analysis was used to genetically map the human OX-2 homologue to chromosome 3. Throughout the period between the cloning of the rat and human OX-2 cDNAs to the present, no function has been ascribed to the OX-2 protein. As recently as 1995, the sequence homology between the Alzheimer amyloid (770) protein and the OX-2 antigen was reported not to be predictive of a function for the OX-2 protein (Richard, S. J. et al., (1995) Brain Res. Bull. 38(3):305-306).

Summary of the Invention

A functional in vivo activity for the OX-2 protein is disclosed herein, namely, the OX-2 protein serves as a T cell costimulatory molecule to modulate a T cell mediated immune response to an antigen in vivo. The T cell costimulatory activity is believed to be mediated by binding of the OX-2 protein to a receptor (the OX-2 receptor) on the target cell surface (e.g., a T cell). Accordingly, functional variants of the OX-2 protein which modulate costimulation via binding to the OX-2 receptor also are provided. Such functional variants include agonists (or antagonists) of the OX-2 protein which mimic (or inhibit) the T cell costimulatory activity of the OX-2 protein. The invention embraces compositions containing the functional variants of the OX-2 protein, nucleic acids encoding the functional variants, vectors containing these nucleic acids, and host cells including the vectors.

According to one aspect of the invention, a method for modulating a T cell mediated immune response in a subject is provided. The subject is a mammal that has been diagnosed as having a condition that is treatable by modulation of the subject's T cell mediated immune response. The method involves administering to the subject a therapeutically effective amount of an OX-2 therapeutic agent to modulate the T cell mediated immune response and thereby treat the condition. As used herein, OX-2 therapeutic agents refers to the OX-2 protein, an OX-2 agonist, an OX-2 antagonist, and other related molecules (e.g., nucleic acids encoding the foregoing proteins, antibodies that react with OX-2 and affect its ability to bind to the OX-2 receptor) that are useful for modulating a T cell mediated immune response.

Conditions that are treatable by up regulation of a T cell mediated immune response include immunosuppressive disorders (e.g., AIDS), neoplastic states and infections using, e.g., vaccine strategies for infectious agents and anti-tumor gene therapy approaches. Conditions that are treatable by down regulation of a T cell mediated immune response include autoimmune diseases (such as rheumatoid arthritis and lupus), graft versus host disease and transplant rejection.

According to yet another aspect of the invention, methods and compositions for diagnosing a condition (e.g., an immunosuppressive disorder) that is treatable by administration of an OX-2 therapeutic agent (e.g., the OX-2 protein, an OX-2 agonist, or an OX-2 antagonist) are provided. The method involves contacting a biological sample of the subject with an OX-2 diagnostic agent to detect the presence of OX-2 (or a nucleic acid encoding OX-2) in the biological sample. OX-2 diagnostic agents include, for example, antibodies which specifically recognize the OX-2 protein, as well as nucleic acids which specifically hybridize to an OX-2 transcript under stringent conditions in vitro and/or which specifically hybridize to an OX-2 transcript in vivo. Diagnostic kits for detecting the presence of an OX-2 protein in a biological sample, as well as vials containing known amounts of the OX-2 protein or nucleic acid for use as standards in the diagnostic kits, also are provided herewith. The diagnostic kits further contain instructions for using the OX-2 diagnostic agents to determine the presence or absence of OX-2 or an OX-2 related molecule (e.g., a nucleic acid encoding OX-2) in a biological sample. In an analogous fashion, diagnostic agents (e.g., an OX-2-immunoglobulin fusion protein) can be provided for detecting the presence of an OX-2 receptor, e.g., for diagnosing a condition that is treatable by administration of an OX-2 receptor by, for example, gene therapy for delivery of the receptor in situ.

The biological sample can be located in vivo (e.g., the biological sample can be a lymphatic tissue), in which instance the OX-2 diagnostic agent is used to detect OX-2 expression in vivo (e.g., for imaging purposes). Alternatively, the biological sample can be located in vitro. For example, the biological sample can be a cell-containing sample, e.g., peripheral blood or biopsy material, in which the cells are, for example, T cells, B cells, thymocytes, endothelial cells, neurons, and dendritic cells, and the presence of OX-2 in the cell-containing sample is determined to diagnose the condition.

Yet another aspect of the invention is directed to a method for enhancing the effectiveness of a vaccine by coadministering OX-2, an OX-2 agonist (or a nucleic acid encoding OX-2 or an OX-2 agonist that can be expressed in vivo) to the recipient of the vaccine. According to this aspect of the invention, enhancing the effectiveness of a vaccine to elicit an immune response to an antigen involves coadministering a therapeutically effective dose of an OX-2 therapeutic agent to the subject to up regulate the T cell mediated immune response of the subject to the antigen. The OX-2 therapeutic agent can be administered simultaneously or sequentially with the vaccine. In a particularly preferred embodiment, the vaccine contains the

antigen, as well as the OX-2 therapeutic agent. For example, a vaccinia virus, genetically engineered to express the antigen, together with the OX-2 therapeutic agent on its surface, can be administered to a subject to elicit an enhanced immune response to the antigen. Optionally, the vaccinia virus can be genetically engineered to further express a major histocompatibility complex molecule (MHC molecule) to obtain a vaccine that (i) expresses the antigen, (ii) presents the antigen on its surface in the context of the MHC molecule, and (iii) delivers the OX-2 therapeutic agent to up regulate the immune response of the subject to the presented antigen. Preferably, the vaccinia virus further is genetically engineered to additionally express other costimulatory molecules and/or cytokines (e.g., IL-12). In an analogous manner, cancer cells which express cancer specific (surface) antigens can be genetically engineered to coexpress an OX-2 therapeutic agent to enhance the T cell mediated immune response of the subject to cancer specific antigens.

Novel compositions containing the OX-2 protein, functional variants of the OX-2 protein which bind to the OX-2 receptor and/or which exhibit an OX-2 T cell costimulatory activity, nucleic acids containing the same, vectors containing the nucleic acids and host cells including the vectors also are provided. Antibodies which specifically recognize the OX-2 protein or its functional variants and/or which inhibit the T cell costimulatory activity of the OX-2 protein or its functional variants also are provided. The invention embraces both soluble and insoluble forms of the OX-2 protein. Soluble forms of the OX-2 protein include, for example, chimeric OX-2 molecules in which one or both of the OX-2 extracellular (immunoglobulin) domain(s) is covalently coupled to yet another soluble protein (e.g., an immunoglobulin heavy chain). The invention also embraces liposome-associated forms of the OX-2 protein or its functional variants in which the OX-2 protein/liposome structure is constructed and arranged to permit contact of the OX-2 extracellular domain(s) with OX-2 receptors that are located on OX-2 targeted cells.

According to a particularly preferred aspect of the invention, a chimeric OX-2 molecule is provided. As used herein, a chimeric OX-2 molecule refers to protein chimeras such as fusion proteins, as well as nucleic acid chimeras which, e.g., encode the protein chimeras. The chimeric OX-2 molecules are useful as OX-2 therapeutic agents and/or diagnostic agents. By analogy to the known T cell costimulatory molecules, it is believed that one or both of the OX-2 extracellular immunoglobulin domains interact with an OX-2 receptor that is present on a target cell (e.g., a T cell) to modulate a T cell mediated immune response. A particularly preferred embodiment of the invention is a chimeric OX-2 molecule in which the extracellular IgV-like

domain and/or the extracellular IgC-like domain of the OX-2 protein are covalently coupled at their C-terminus to the N-terminus of an immunoglobulin constant region. The chimeric OX-2 protein binds to the OX-2 receptor and exhibits an OX-2 T cell costimulatory activity. The chimeric OX-2 protein, as well as the other functional variants of OX-2, do not bind to CTLA4 and CD28.

Exemplary OX-2 T cell costimulatory activities include the ability to costimulate CD4+ T cells and/or the ability to costimulate thymocytes in an antigen dependent context (see, e.g., the Examples). Thus, as would be immediately apparent to one of ordinary skill in the art, novel OX-2 therapeutic agents (e.g., chimeric OX-2 proteins) that function as OX-2 T cell costimulatory molecules (i.e., functional variants of OX-2) can be identified using no more than routine experimentation by, for example, making the putative OX-2 therapeutic agents (e.g., using routine recombinant methods) and screening the agents in in vitro screening assays to identify those agents having a T cell costimulatory activity (to identify OX-2 protein agonists) and/or agents having the ability to inhibit OX-2 T cell costimulation (to identify OX-2 protein antagonists).

According to yet another aspect of the invention, isolated novel proteins encoded by the OX-2 gene are provided. The proteins correspond to alternative forms of the OX-2 protein which bind to an OX-2 receptor and exhibit a T cell costimulatory activity. Preferably, the alternative forms correspond to naturally-occurring, alternatively-spliced forms of the OX-2 protein or to functional variants of the alternatively-spliced forms that can be produced using recombinant DNA techniques. By analogy to the B7 family of T cell costimulatory molecules, it is believed that alternatively-spliced forms of the OX-2 T cell costimulatory molecules exist in nature. Exemplary novel functional variants of the OX-2 protein contain an alternative structural domain (i.e., a structural domain having an amino acid sequence which differs from that reported for the OX-2 protein, e.g., a novel signal sequence) or have a structural domain deleted (e.g., the IgC-like domain deleted). Exemplary novel functional variants of the OX-2 nucleic acids (e.g., an OX-2 cDNA, an OX-2 mRNA) have a nucleotide sequence which differs from that reported for the OX-2 nucleic acid in having an exon deleted or replaced by a novel exon encoding a novel structural domain. Confirmation of the predicted occurrence in nature of the alternatively-spliced forms of the OX-2 protein and/or nucleic acid will further support additional functional roles for the OX-2 protein in vivo. For example, we believe that the interaction between the OX-2 protein on one cell and an OX-2 receptor expressed on another cell (e.g., a T cell) likely

involves bidirectional signal transduction between the cells (rather than unidirectional signal transduction to the cell expressing the OX-2 receptor).

According to another aspect of the invention, OX-2 functional variants which bind to the OX-2 receptor and exhibit an OX-2 T cell costimulatory activity and which further contain a novel signal peptide domain are provided. The disclosure herein of a function for the OX-2 protein permits the identification of novel OX-2 T cell costimulatory molecule genes which contain exons encoding different signal peptide domains that can be used in an alternate manner. By analogy to the B7-2 protein, it is believed that alternative splicing of the mRNA transcripts of the OX-2 gene results in the generation of naturally-occurring OX-2 T cell costimulatory molecules having different signal peptide domains. Confirmation of the existence of alternative signal peptide domain forms of the OX-2 protein will further support alternative functional roles for the signal peptide of the OX-2 protein.

Still another aspect of the invention is directed to novel OX-2 protein functional variants that bind to the OX-2 receptor and exhibit an OX-2 T cell costimulatory activity and in which at least one structural domain of the known OX-2 proteins is deleted. Isolated nucleic acids encoding the foregoing OX-2 protein functional variants also are provided. In one preferred embodiment, the OX-2 protein has an IgC-like domain deleted (i.e., an immunoglobulin variable-like domain is linked directly to a transmembrane/cytoplasmic domain). In another preferred embodiment, the OX-2 protein has an IgV-like domain deleted (i.e., a signal peptide domain is linked directly to an immunoglobulin constant-like domain).

An isolated nucleic acid molecule of the invention can be incorporated into a recombinant expression vector and transfected or otherwise delivered into a host cell to express a novel structural form of the OX-2 protein. The isolated nucleic acids of the invention can further be used to create transgenic and homologous recombinant non-human animals. The novel OX-2 T cell costimulatory molecules (proteins and nucleic acids) provided by the invention can be used (1) to modulate a costimulatory signal in a T lymphocyte; (2) to raise antibodies against novel structural domains (or exons) of the OX-2 protein (or nucleic acid); (3) to identify a receptor for the OX-2 protein; (4) to identify agents which stimulate the expression of alternative forms of the OX-2 T cell costimulatory molecules; (5) to identify components of the signal transduction pathway induced in a cell expressing a costimulatory molecule in response to an interaction between the costimulatory molecule and its receptor on a T lymphocyte or other cell type and/or (6) to create mouse models of OX-2 to further elucidate OX-2 function.

These and other aspects of the invention, as well as various advantages and utilities, will be more apparent with reference to the drawings and the detailed description of the preferred embodiments.

All references, patents, patent applications and patent publications identified herein are incorporated in their entirety herein by reference.

Brief Description of the Drawings

Figure 1 shows the FACS analysis of the CHO cell transfectants. The histograms confirm the expected phenotype of the CHO cell transfectant used in the study. The symbols refer to strong positive stain (++), weak positive stain (+), and negative stain (-).

Figure 2A shows the costimulation of T cell proliferation using Balb/c responder T cells and anti-CD3. Paraformaldehyde fixed CHO cells were used to provide costimulation to CD4+ splenic T cells from "old" (52 weeks) or "young" mice (6-8 weeks) which had been activated with suboptimal concentrations of anti-CD3 (at 1 μg/ml). CHOmurine-IAd-B7-1 stimulated equivalent levels of proliferation in "old" and "young" T cells while OX-2 showed a two fold greater activity with the "old" T cells compared to "young" T cells. Note that the IAd is irrelevant for purposes of this experiment.

Figure 2B shows the specificity of costimulation of T cell proliferation by ratOX-2. Paraformaldehyde fixed CHO-IAd-ratOX-2 cells were used to provide costimulation to CD4+ splenic T cells from young mice (6-8 weeks) which had been activated with anti-CD3 at 1 μg/ml. CHO cells were preincubated with the indicated blocking antibody reagents for 30 minutes prior to the addition of T cells and anti-CD3. Proliferation was measured at day 3 with tritium incorporation. Note that the IAd is irrelevant for purposes of this experiment.

Figure 2C shows the costimulation of T cell proliferation using T cells from the T cell receptor (TCR) transgenic strain that contains rearranged TCR α and β in the germ line DNA that encode a TCR specific for chicken ovalbumin 323-339 bound to the I-Ad class II MHC molecule. Paraformaldehyde fixed CHO cells were used to provide costimulation to CD4+ splenic T cells from DO.11 mice which had been activated with OVA-peptide at 10 μg/ml. Both CHO-IAd-B7-1 and OX-2 showed costimulatory activity in an antigen-dependent fashion. A direct comparison between the costimulatory potential of B7-1 and ratOX-2 is not implied by these proliferation data as that would require further information regarding antigen density on the CHO cells as well as receptor density on the T cell.

Figure 3 shows the costimulation of thymocytes. Paraformaldehyde fixed CHO cells

were used to provide costimulation to thymocytes from DO.11 mice which had been activated with nothing, anti-CD3 at 1 μg/ml or OVA-peptide at 10 μg/ml. CHO-IAd-ratOX-2 showed the costimulatory activity in both instances.

Detailed Description of the Invention

Methods and compositions for modulating (up regulating or down regulating) a T cell mediated immune response to an antigen in a subject in which the subject has a condition that is treatable by modulation of the subject's T cell mediated immune response are described herein. Exemplary conditions that are treatable by up regulation of a T cell mediated immune response include immunosuppressive disorders, (e.g., AIDS), neoplastic states and infections using, e.g., vaccine strategies for infectious agents and anti-tumor gene therapy approaches. Exemplary conditions that are treatable by down regulation of a T cell mediated immune response include autoimmune diseases (such as rheumatoid arthritis and lupus), graft versus host disease and transplant rejection. Thus, the invention can be used to augment a weak immune response to an antigen (e.g., to enhance the effectiveness of a vaccine) or to suppress a harmful (e.g., autoimmune) immune response in a subject.

The methods of the invention involve administering to the subject a therapeutically effective amount of an OX-2 therapeutic agent to modulate the T cell mediated response and thereby treat the condition. As used herein, "OX-2 therapeutic agent" refers to the OX-2 protein, OX-2 agonists, OX-2 antagonists, and related molecules (e.g., nucleic acids encoding the foregoing proteins) that are useful for modulating a T cell mediated immune response in vivo. OX-2 agonists (or OX-2 antagonist) are identified in screening assays which detect the ability of a putative agonist (or antagonist) to costimulate (or compete with the ability of OX-2 or an OX-2 agonist to costimulate) a T cell response in vitro and/or in vivo. Screening assays which can be used to measure the functional activity of a putative OX-2 agonist or OX-2 antagonist are provided in the Examples. In general, the screening assays measure the ability of OX-2 or an OX-2 agonist to (1) costimulate CD4+ T and/or CD8+ T cells and/or (2) costimulate thymocytes in an antigen dependent context. Alternatively, the screening assays measure the ability of a putative OX-2 antagonist to inhibit the above-described OX-2 (or OX-2 agonist) costimulatory activity. The OX-2 agonists and OX-2 antagonists do not bind to CTLA4 and CD28.

OX-2 agonists are structural variants of OX-2 which mimic the functional activity of the naturally-occurring OX-2 protein, i.e., the OX-2 agonists are functional and structural variants of the naturally-occurring OX-2 protein. Presumably, the OX-2 agonists costimulate T cells by

interacting with an OX-2 receptor that is expressed on the surface of the OX-2 targeted cell (e.g., a T cell). The most preferred OX-2 agonists are represented by soluble functional variants of the OX-2 protein. Exemplary soluble OX-2 agonists include (1) functional variants of the OX-2 protein in which one or more structural domains of the OX-2 protein have been deleted, (2) functional variants of the OX-2 protein containing only the OX-2 extracellular domain (or portions thereof which bind to the OX-2 receptor), and (3) chimeric OX-2 proteins in which one or more extracellular domains of the OX-2 protein are covalently coupled to a soluble polypeptide (e.g., an immunoglobulin constant region). In the preferred embodiments, the OX-2 agonist is a chimeric OX-2 protein that includes the IgV-like domain (denoted domain "B" herein) and/or the IgC-like domain (denoted domain "C" herein) coupled to an immunoglobulin constant region amino acid sequence. The more preferred OX-2 agonists include the entire IgV-like and/or IgC-like domains. Alternative preferred OX-2 agonists are or include (i.e., if a chimeric molecule) functionally active portions of the IgV-like and/or IgC-like domain, i.e., the portions are of a sufficient size and composition to costimulate T cells in the above-noted screening assays. In the most preferred embodiments, the OX-2 agonist includes the IgV ("B") domain and may include one or more other portions of the OX-2 protein (e.g., the IgC-like domain, the signal sequence, the transmembrane and cytoplasmic domain). Preferably, the portions of the IgV-like and IgC-like domain that can costimulate T cells are "unique fragments." A "unique fragment" of a protein or nucleic acid sequence is a fragment which is not currently known to occur elsewhere in nature (except in allelic or allelomorphic variants). Unique fragments act as a "signature" of the gene or protein from which they are derived. A unique fragment will generally exceed 15 nucleotides or 5 amino acids in length. One of ordinary skill in the art can readily identify unique fragments of the OX-2 protein or nucleic acid by searching available computer databases of nucleic acid and protein sequences such as Genbank, (Los Alamos National Laboratories, USA), EMBL, or SWISS-PROT. A unique fragment is particularly useful, for example, in generating monoclonal antibodies or in screening genomic DNA or cDNA libraries.

The invention also embraces insoluble OX-2 agonists and antagonists. A representative insoluble OX-2 agonist is a liposomal formulation in which the OX-2 protein (or a functional variant thereof)/liposome is constructed and arranged to permit contact of the OX-2 extracellular domain(s) (or functionally active portions thereof) with, e.g., an OX-2 receptor located on an OX-2 targeted cell. Exemplary protocols for liposome formulations, e.g., for in vivo delivery,

are described in U.S. Patent Nos. 4,921,757 (issued to Wheatley et al.); 4,925,661 (issued to Huang et al.); 5,225,212 (issued to Martin et al.); EP256989 (Fidler et al.); and PCT/US94/08568 (Tari et al.).

According to yet another aspect of the invention, the OX-2 agonist is a chimeric OX-2 protein (i.e., a fusion protein) that is encoded by a chimeric OX-2 nucleic acid. Preferably, the chimeric OX-2 agonist is a chimeric immunoglobulin polypeptide which includes (a) at least one amino acid sequence selected from the group consisting of the IgV-like domain (domain "B") and the IgC-like domain (domain "C") of OX-2 and (b) an immunoglobulin constant region amino acid sequence. The OX-2 IgV-like and/or IgC-like domain is coupled at its C-terminus to the N-terminus of the immunoglobulin constant region amino acid sequence. Exemplary immunoglobulin constant region amino acid sequences that are suitable for forming chimeric OX-2 agonists (and antagonists) are described in U.S. Patent No. 5,428,130, issued to Capon et al.

In addition to the above-noted therapeutic uses, OX-2 fusion proteins are useful as reagents for identifying the predicted OX-2 receptor on a variety of cell types. For example, soluble OX-2 fusion proteins containing one or more extracellular domains (or functionally active portions thereof) of the OX-2 protein can be labeled with a detectable label (e.g., a radioactive, colorimetric, fluorometric label) and used to detect the presence of the predicted OX-2 receptor on cells in vitro or in vivo. In this manner, the chimeric OX-2 proteins can be used to screen different populations of T cells to determine which cell populations express a receptor for OX-2 and thereafter, to clone the receptor from the selected T cell populations. See Examples.

OX-2 antagonists are agents which neutralize or otherwise impede (e.g., competitively inhibit) the functional activity of the OX-2 protein. Exemplary OX-2 antagonists include OX-2 protein antagonists, OX-2 oligonucleotide antagonists and OX-2 antibody antagonists. The first class of OX-2 antagonists are structural variants of the OX-2 protein ("OX-2 protein antagonists") which inhibit the functional activity of OX-2 by, e.g., competitively inhibiting the binding of the protein (or OX-2 agonists) to the OX-2 receptor. The most preferred OX-2 protein antagonists are soluble forms of the OX-2 protein which have been modified so that the antagonist binds to the OX-2 receptor but does not exhibit a T cell costimulatory activity. Typically, receptors for T cell costimulatory molecules are activated to exhibit a T cell costimulatory activity by crosslinking to a multivalent ligand (OX-2). For example, an OX-2 antagonist can be a single chain OX-2 fusion protein in which OX-2 is coupled to an

immunoglobulin heavy chain, wherein the OX-2 portion of the chimeric molecule has been modified to prevent crosslinking of the chimeric molecule to the OX-2 receptor. Thus, in its broadest sense, an OX-2 antagonist is OX-2 or an OX-2 agonist which has been modified to prevent crosslinking of the OX-2 receptor. Such modifications include, but are not limited to, making conservative amino acid substitutions (e.g., alanine or serine substituted for cysteine) in the hinge region of the OX-2 Ig fusion protein such that only monomeric proteins are generated which are incapable of crosslinking the OX-2 receptor. Thus, the modifications embraced by the instant invention are modifications that are intended to convert a multivalent OX-2 ligand to a monovalent OX-2 ligand and thereby prevent crosslinking of the OX-2 receptor upon OX-2 engagement. Exemplary OX-2 protein antagonists include fragments of the OX-2 protein extracellular domain which interact with the OX-2 receptor (as assessed in, e.g., a receptor binding assay) but which do not exhibit a T cell costimulatory activity (as assessed in, e.g., an in vitro screening assay). The OX-2 antagonists are useful for down regulating a T cell mediated immune response, for example, in treating an autoimmune condition.

The second class of OX-2 antagonists ("OX-2 oligonucleotide antagonists") specifically inhibit the transcription or translation of the OX-2 protein (e.g., antisense sequences that specifically hybridize to an OX-2 DNA or OX-2 mRNA, ribozymes that specifically cleave the OX-2 transcript). OX-2 oligonucleotide antagonists are initially identified in in vitro screening assays ("pre-screening" assays) which measure the ability of the putative oligonucleotide antagonists to specifically hybridize to OX-2 mRNA under stringent conditions or to cleave the OX-2 mRNA under ribozyme cleavage conditions (see, e.g., U.S. Patent No. 4,987,071 (issued to Cech et al.) and PCT/US94/06316 (applicant Ribozyme Pharmaceuticals, Inc.). In general, the OX-2 oligonucleotide antagonists which hybridize to the OX-2 mRNA contain between about six and 100 bases, preferably between about 30 and 50 bases, and have a nucleotide sequence which is complementary to the nucleic acid sequence encoding, e.g., unique portions of the extracellular domain of the OX-2 protein or its signal sequence. In a particularly preferred embodiment, the OX-2 oligonucleotide antagonists have a nucleotide sequence that is complementary to the nucleotide sequence encoding amino acids -19 to -13 (Clark et al., EMBO 4(1):113-118, 1985, numbering scheme) of the OX-2 protein. For example, the nucleotide sequence, 5'-CAG GCT GTA GGT GGA CAG ATG-3' (SEQ ID NO: 13), which is one hundred percent complementary to the nucleotide sequence encoding the OX-2 signal sequence can be used to inhibit the translation of the OX-2 mRNA. Following pre-screening, the putative OX-2

oligonucleotide antagonists are screened in functional activity assays to identify antagonists which inhibit OX-2 (or OX-2 agonist) mediated T cell costimulation in, for example, an animal model, in which the ability of OX-2 to costimulate T cells in vivo is determined or in an in vitro assay which measures the ability of the putative antagonists to inhibit OX-2 expression on cells which normally express OX-2 in vitro.

As used herein, the term "isolated" in reference to an oligonucleotide, means an RNA or DNA polymer, portion of genomic nucleic acid, cDNA or synthetic nucleic acid which, by virtue of its origin or manipulation: (a) is not associated with all of a nucleic acid with which it is associated in nature (e.g., is present in a host cell as a portion of an expression vector); or (b) is linked to a nucleic acid or other chemical moiety other than that to which it is linked in nature; or (c) does not occur in nature. By "isolated" it is further meant a nucleic acid sequence: (i) amplified in vitro by, for example, the polymerase chain reaction (PCR); (ii) synthesized by, for example, chemical synthesis; (iii) recombinantly produced by cloning; or (iv) purified from a more complex molecule or from a mixture of molecules, such as by cleavage and size fractionation. Due to the degeneracy of the genetic code, many different oligonucleotide sequences can be identified which encode SEQ ID NO: 2. Accordingly, the invention embraces oligonucleotides which encode the extracellular domain (as well as its fragments) but which have nucleotide sequences which differ from the sequences of the naturally-occurring OX-2 gene.

As used herein, the phrase "hybridizing under stringent conditions" is a term of art which refers to the conditions of temperature and buffer components which will permit hybridization of a particular oligonucleotide or nucleic acid to its complementary sequence and not to non-complementary sequences. The exact conditions which constitute "stringent" conditions, depend upon the length of the nucleic acid sequence and the frequency of occurrence of subsets of that sequence within other non-identical sequences. By varying hybridization conditions from a level of stringency at which no hybridization occurs to a level at which hybridization is first observed, one of ordinary skill in the art can, without undue experimentation, determine conditions which will allow a given sequence to hybridize only with identical sequences. Suitable ranges of such stringency conditions are described in Krause, M.H. and S.A. Aaronson, Methods in Enzymology, 200:546-556 (1991). Stringent hybridization conditions, depending upon the length and commonality of a sequence, may include hybridization conditions of from 30 to 60°C and from 5x to 0.1x SSC. Highly stringent hybridization conditions may include hybridization at 45°C and 0.1 SSC. Less than stringent conditions are employed to isolate nucleic acid sequences

which are substantially similar, allelic or homologous to any given sequence. In a particularly preferred embodiment, the isolated oligonucleotide is an antisense oligonucleotide that is 100% complementary to the above-identified nucleotide sequence encoding amino acids -19 to -13 (Clark et al, ibid.) of the OX-2 protein (i.e., the shared signal sequence). Exemplary high stringency hybridization conditions are provided in U.S. Patent Application Serial No. 08/199,776, the contents of which are incorporated herein by reference.

Alternatively, the isolated oligonucleotide is an antisense oligonucleotide (e.g., SEQ. ID NOS: 16 or 17) that is capable of hybridizing under stringent conditions to a unique fragment of the OX-2 extracellular domain, e.g., a unique fragment of the IgV-like domain. The preferred antisense nucleic acids hybridize under stringent conditions to a unique fragment of the IgV-like domain. Exemplary antisense oligonucleotides which hybridize under stringent conditions to the IgV-like domain of OX-2 include:

SEQ ID NO:16 (5'- TTG TTC ATC CTG GGT CAC CAC TTC CAC TTG -3'); and

SEQ ID NO:17 (5'- CTG GGT CAC CAC TTC CAC TTG -3').

As used herein, the phrase "unique fragment" refers to a nucleic acid sequence having less than 25% sequence homology with previously identified nucleic acid sequences. More preferably, the unique fragments have less than 10% sequence homology with known nucleic acid sequences. Such unique fragments can be identified by searching the Genbank, PIR and/or Swiss-Prot data bases using the Eugene program available through the Harvard Molecular Biology Core Research Resource, Cambridge, MA. The unique fragments are useful, for example, as probes and primers in nucleic acid hybridization assays and in amplification reactions, respectively.

A third class of OX-2 agonists are antibodies to the OX-2 protein ("OX-2 antibody antagonists"). The antibodies recognize the extracellular domain of the OX-2 protein and are capable of specifically inhibiting the binding of OX-2 or of an OX-2 agonist to the OX-2 receptor and thereby inhibiting the functional activity of OX-2 or the OX-2 agonist. Exemplary OX-2 antibody antagonists, some of which are commercially available, are described in the Examples. Despite the existence of commercially available antibodies to OX-2, the use of such antibodies to modulate a T cell mediated immune response previously has not been possible because of the lack of understanding of a functional role for OX-2. Thus, the instant disclosure of a functional role for OX-2 permits the design and construction of antibodies which specifically bind to the extracellular domain of OX-2 in a manner to modulate an OX-2 T cell mediated immune response. In particular, the instant disclosure permits the design of antibody fragments,

as well as humanized monoclonal antibodies, for modulating an OX-2 T cell mediated immune response. Absent a knowledge of a functional role for OX-2, one skilled in the art would not have been motivated to design and construct antibodies which function as OX-2 antibody antagonists, and in particular, one skilled in the art would not have been motivated to make humanized antibodies to the OX-2 protein for human therapeutic applications.

In addition to therapeutic applications, OX-2 specific antibodies are useful for diagnosing condition(s) that are treatable by administration of an OX-2 therapeutic agent. The antibodies to OX-2 also are useful in combination with FACS analysis for the identification of functionally important subpopulations of T cells for which only "surrogate" markers may be available. Accordingly, the ability to use antibodies to OX-2 as possible memory T cell markers permits one skilled in the art to diagnose a condition that is manifested by a defect in the ability to make memory T cells for modulation of a T cell mediated immune response.

The OX-2 therapeutic agent is administered to the subject to treat a condition that is treatable by modulation of the subject's T cell mediated immune response. A therapeutically effective amount of the OX-2 therapeutic agent is administered to the subject in accordance with standard medical practice for administering a protein or nucleic acid agent. Exemplary methods for administration of a therapeutic agent, including calculation of dosages and the preparation of various formulations are provided in Remington's Pharmaceutical Sciences, 18th edition, 1990. (See, also, U.S. Patent No. 5,428,130). Exemplary methods for delivering a nucleic acid to a somatic cell for human gene therapy are provided in U.S. Patent No. 5,399,346, issued to Anderson et al. and PCT Application No.PCT/US94/06809 (WO95/00654).

As used herein, a "therapeutically effective amount" of an OX-2 therapeutic agent of the invention is a dosage that is large enough to produce the desired effect in which the symptoms of the condition are ameliorated or prevented but is not so large as to cause adverse side effects. Generally, a therapeutically effective amount varies with the subject's age, condition, and sex, as well as with the extent of the disease in the subject and can be determined by one of skill in the art. The dosage may be adjusted by the individual physician or veterinarian in the event of any complication. Typically, a therapeutically effective amount is between about 0.01 mg/kg to about 500 mg/kg, preferably between about 0.1 mg/kg to about 200 mg/kg, most preferably between about 0.2 mg/kg to about 20 mg/kg, in one or more dose administrations daily, for one or several days. The OX-2 therapeutic agents of the invention can be administered by, for example, injection or by gradual infusion over time. For example, the administration of the

monoclonal antibodies of the invention may, for example, be intravenous, intraperitoneal, intramuscular, intra cavity, subcutaneous, or transdermal. Those of skill in the art can readily determine the various parameters and conditions for preparing pharmaceutical preparations containing an OX-2 therapeutic agent in a pharmaceutically acceptable carrier (e.g., normal saline) and administering the preparation to the subject. See, e.g., Remington's Pharmaceutical Sciences, 18th edition, 1990, pp 1694-1712.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

In general, the therapeutically effective amount is between about 1 μg and about 100 mg/kg. The preferred amount can be determined by one of ordinary skill in the art in accordance with standard practice for determining optimum dosage levels of the agent. The OX-2 therapeutic agents are formulated into a pharmaceutical composition by combination with an appropriate pharmaceutically acceptable carrier. For example, OX-2, the OX-2 agonists or OX-2 antagonists may be used in the form of their pharmaceutically acceptable salts, or may be used alone or in appropriate association, as well as in combination with other pharmaceutically active compounds. The OX-2 agonists or antagonists may be formulated into preparations in solid, semisolid, or liquid form such as tablets, capsules, powders, granules, ointments, solutions, suppositories and injections, in usual ways for oral, parenteral, or surgical administration. Exemplary pharmaceutically acceptable carriers are described in U.S. 5,211,657, the entire contents of which patent are incorporated herein by reference. The invention also includes locally administering the composition as an implant.

According to a related aspect of the invention, a method for enhancing, in a subject, the effectiveness of a vaccine is provided. According to this aspect of the invention, the effectiveness of a vaccine is enhanced by coadministering to the subject a therapeutically effective dose of an OX-2 therapeutic agent (OX-2 or an OX-2 agonist) to up regulate the T cell

mediated immune response of the subject to an antigen. In a particularly preferred embodiment, the vaccine includes the antigen together with the OX-2 therapeutic agent. For example, the vaccinia virus can be genetically engineered to express the antigen, together with the OX-2 therapeutic agent on its surface, and can be used as a vaccine to induce an immune response to the antigen. Optionally, the vaccinia virus can be genetically engineered to further express a major histocompatibility complex molecule (MHC molecule) or other costimulatory molecule and/or cytokine (e.g., IL-12) on its surface to result in a vaccine that is capable of (i) expressing the antigen, (ii) presenting the antigen on its surface in the context of the MHC molecule, and (iii) delivering the OX-2 therapeutic agent to up regulate the immune response of the subject to the presented antigen. Thus, the invention also provides a vaccine which contains the OX-2 protein and/or its functional equivalent (e.g., an OX-2 agonist).

According to another aspect of the invention, methods for diagnosing in a subject, a condition that is treatable by administration of an OX-2 therapeutic agent, are provided. The methods involve contacting a biological sample of the subject with an OX-2 diagnostic agent to detect the presence of OX-2 in the biological sample. As used herein, "contacting" means placing the biological sample in sufficient proximity to the OX-2 diagnostic agent and under the appropriate conditions of, e.g., concentration, temperature, time, ionic strength, to allow the specific interaction between the diagnostic agent and OX-2 proteins (or nucleic acids encoding said proteins) that are present in the biological sample. In general, the conditions for contacting the diagnostic agent with the biological sample are conditions known by those of ordinary skill in the art to facilitate a specific interaction between a molecule and its cognate (e.g., a protein and its receptor cognate, an antibody and its protein antigen cognate, a nucleic acid and its complementary sequence cognate) in a biological sample. Exemplary conditions for facilitating a specific interaction between a molecule and its cognate are described in U.S. Patent No. 5,108,921, issued to Low et al.

The biological sample can be located in vivo or in vitro. For example, the biological sample can be a lymphatic tissue in vivo and the OX-2 diagnostic agent can be used to detect the presence of OX-2 in the lymphatic tissue (e.g., for imaging portions of the lymphatic tissue that express the OX-2 protein). Alternatively, the biological sample can be located in vitro (e.g., a blood sample, tissue biopsy, tissue extract). In a particularly preferred embodiment, the biological sample can be a cell-containing sample, more preferably a sample containing T cells (e.g., a peripheral blood sample), endothelial cells, B cells, dendritic cells or neurons.

In general, the OX-2 diagnostic agents include many of the same types of agents which are embraced by the phrase "OX-2 therapeutic agents". Typically, the OX-2 therapeutic agents further include a detectable label to detect the presence of the naturally-occurring OX-2 protein or nucleic acid encoding the naturally-occurring OX-2 protein. Thus, for example, the OX-2 diagnostic agent can be a labeled oligonucleotide which hybridizes under stringent conditions to an OX-2 mRNA. Exemplary stringent conditions for in vitro assays are known in the art. As discussed above, "stringent hybridization conditions" is a term of art understood by those of ordinary skill in the art. For any given nucleic acid sequence, stringent hybridization conditions are those conditions of temperature and buffer solution which will permit hybridization of that nucleic acid sequence to its complementary sequence and not to substantially different sequences. The exact conditions which constitute "stringent" conditions, depend upon the length of the nucleic acid sequence and the frequency of occurrence of subsets of that sequence within other non-identical sequences. By varying hybridization conditions from a level of stringency at which no hybridization occurs to a level at which hybridization is first observed, one of ordinary skill in the art can, without undue experimentation, determine conditions which will allow a given sequence to hybridize only with identical sequences. Suitable ranges of such stringency conditions are described in Krause, M.H. and S.A. Aaronson, Methods in Enzymology 200:546-556 (1991). Stringent hybridization conditions, depending upon the length and commonality of a sequence, may include hybridization conditions of 30°C-65°C and from 5X to 0.1X SSPC. Less than stringent hybridization conditions are employed to isolate nucleic acid sequences which are substantially similar, allelic or homologous to any given sequence.

Novel Structural Embodiments

The following description sets forth illustrative embodiments of the invention and is not intended to limit the scope of the invention in any way.

According to one aspect of the invention, functional variants of an OX-2 T cell costimulatory molecule ("OX-2 functional variants") are provided. The functional variants have a novel structural form that is related to the structure of the naturally-occurring OX-2 protein or nucleic acid. According to one embodiment, the OX-2 functional variants have an OX-2 structural domain deleted. According to another embodiment, the OX-2 functional variant has a structure which corresponds to that of a naturally-occurring, alternatively-spliced form ("isoform") of the OX-2 protein. The alternatively-spliced OX-2 protein is isolated from a natural source or alternatively, is produced using standard recombinant DNA techniques.

Functional variants of the OX-2 proteins share common structural and functional properties with the OX-2 protein. Thus, the OX-2 functional variants bind to the OX-2 receptor and exhibit an OX-2 T cell costimulatory activity.

An OX-2 T cell costimulatory activity is determined in a screening assay by measuring the ability of, for example, a putative OX-2 agonist to costimulate CD4+ T cells and/or to costimulate thymocytes in an antigen dependent context. Putative OX-2 antagonists are identified in the screening assays by measuring the ability of the putative OX-2 antagonist to inhibit the costimulatory activity of OX-2 or of a known OX-2 agonist. Exemplary screening assays are provided in the Examples and can be performed by one of ordinary skill in the art using no more than routine experimentation.

Typically, an OX-2 T cell costimulatory molecule of the invention includes the following structural domains: a signal peptide domain (e.g., exon 1), an immunoglobulin variable region-like domain (IgV-like) (e.g., exon 2), an immunoglobulin constant region-like domain (IgC-like) (e.g. exon 3), and a transmembrane/cytoplasmic domain (e.g., exon 4). The OX-2 T cell costimulatory molecule gene is a member of the immunoglobulin gene superfamily and accordingly, the terms "immunoglobulin variable region-like domain" and "immunoglobulin constant region-like domain" as used herein have their art-recognized meanings. Thus, the terms "IgV-like" and "IgC-like" domains refer to protein domains which are homologous in sequence to an immunoglobulin variable region or an immunoglobulin constant region, respectively. For a discussion of the immunoglobulin gene superfamily and a description of IgV-like and IgC-like domains see Hunkapiller, T. and Hood, L. (1989) Advances in Immunology 44:1-63.

Each structural domain of a protein usually is encoded in genomic DNA by at least one exon. Selected aspects of the invention are based, at least in part, on the premise that, to a certain extent, the OX-2 protein gene structure parallels that of the B7 T cell costimulatory molecules described in international application PCT/US95/02576, and the prediction (based upon similarities to the B7 molecules) of the existence of alternative splicing in the OX-2 T cell costimulatory molecule gene which results in novel, naturally-occurring forms of the OX-2 protein. As discussed in international application PCT/US95/02576, exons encoding different forms (isoforms) of a structural domain of a B7 T cell costimulatory molecule can be assembled in an alternative manner by alternative splicing of the B7 primary mRNA transcripts. Alternative-splicing is an art-recognized term which refers to the mechanism by which primary mRNA transcripts of a gene are processed to produce different mature mRNA transcripts

encoding different proteins. Alternative-splicing can result in the excision of different exonic sequences from different primary transcripts. As a result, mature mRNA transcripts can be produced from a single gene that contains different exonic sequences to produce proteins having different amino acid sequences. The terms "alternative forms," "isoforms" or "novel forms" of the OX-2 T cell costimulatory molecule refer to the gene products of a single gene which differ in nucleotide or amino acid sequence from previously disclosed forms of the OX-2 protein produced from the same gene. The invention embraces these alternative nucleic acids, isolated from a natural source or prepared using recombinant techniques, as well as the OX-2 proteins encoded thereby.

The various aspects of this invention are described in detail in the following subsections. Forming part of the present disclosure is the following abbreviated Sequence Listing.

SEQ ID NO:1 is the nucleotide sequence containing exon 1 of the genomic human OX-2 DNA, as described in McCaughan et al., Immunogenetics 25:329-335, 1987.

SEQ ID NO:2 is the amino acid sequence of human OX-2 protein, encoded by exons 1-2-3-4, as described in McCaughan et al., Immunogenetics 25:329-335, 1987.

SEQ ID NO:3 is the nucleotide sequence of rat OX-2 cDNA, exons 1-2-3-4, as described in Clark et al., EMBO 4(1):113-118, 1985.

SEQ ID NO:4 is the amino acid sequence of rat OX-2 protein, encoded by exons 1-2-3-4, as described in Clark et al., EMBO 4(1):113-118, 1985.

SEQ ID NO:5 is the nucleotide sequence of mouse OX-2 cDNA, exons 1-2-3-4 (partial sequence).

SEQ ID NO:6 is the amino acid sequence of mouse OX-2 protein encoded by exons 1-2-3-4 (partial sequence).

SEQ ID NO:7 is the PCR primer O-228.

SEQ ID NO:8 is the PCR primer O-229.

SEQ ID NO:9 is the amino acid sequence of mB7-1 as disclosed in Freeman et al., J. Immunology 143:2714-2722, 1989.

SEQ ID NO:10 is the amino acid sequence of mB7-2 as disclosed in Freeman et al., J. Exp. Med. 178:2185-2192, 1993.

SEQ ID NO:11 is the nucleotide sequence containing exon 2 of the genomic human OX-2 DNA, as described in McCaughan et al., Immunogenetics 25:329-335, 1987 (see also Figure 7).

SEQ ID NO:12 is the nucleotide sequence containing exon 3 of the genomic human OX-2 DNA, as described in McCaughan et al., Immunogenetics 25:329-335, 1987.

SEQ ID NO:13 is the nucleotide sequence (antisense) that is complementary to the nucleic acid encoding the OX-2 signal sequence (5'-CAG GCT GTA GGT GGA CAG ATG-3').

SEQ ID NO:14 is Primer 1 of Example 3 (5'- GAA GTG GTG ACC CAG GAT GAA -3').

SEQ ID NO:15 is Primer 2 of Example 3 (5'- GTA TAA TAA GAT GGA GAT CAA -3').

SEQ ID NO:16 is the nucleotide sequence (antisense) that is complementary to a unique fragment of the IgV-like domain of OX-2 (5'- TTG TTC ATC CTG GGT CAC CAC TTC CAC TTG -3').

SEQ ID NO:17 is another nucleotide sequence (antisense) that is complementary to a unique fragment of the IgV-like domain of OX-2 (5'- CTG GGT CAC CAC TTC CAC TTG -3').

SEQ ID NO:18 is the amino acid sequence of a chicken ovalbumin peptide, OVA-p (cOVA 323-339) which is an IAd restricted peptide antigen (NH2-ISQAVHAAHAEINEAGR-COOH).

SEQ ID NO:19 is the nucleotide sequence containing exon 4 of the genomic human OX-2 DNA, as described in McCaughan et al., Immunogenetics 25:329-335, 1987.

SEQ ID NO:20 is the PCR primer 309 of Example 4.

SEQ ID NO:21 is the PCR primer 226 of Example 4.

SEQ ID NO:22 is the PCR primer FB23 of Example 4.

SEQ ID NO:23 is the amino acid sequence at the hOX-2:Ig junction of the hOX-2-Ig fusion protein of Example 4.

I. Isolated Nucleic Acid Molecules Encoding OX-2 T Cell Costimulatory Protein Molecules

The invention provides an isolated nucleic acid molecule encoding a novel structural form of an OX-2 T cell costimulatory molecule. The novel OX-2 nucleic acid molecules disclosed herein can be isolated from a natural source or produced using recombinant methods. The term "isolated" as used herein in reference to a nucleic acid refers to a nucleic acid that is substantially free of cellular material or culture medium when produced by recombinant DNA

techniques, or chemical precursors or other chemicals when chemically synthesized. An "isolated" nucleic acid is also free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the organism from which the nucleic acid is derived. The term "nucleic acid" is intended to include DNA and RNA and can be either double stranded or single stranded. Preferably, the isolated nucleic acid molecule is a cDNA.

A. Nucleic Acids Encoding Novel Signal Peptide Domains

Alternative B7 mRNA transcripts occur in nature and encode different signal peptide domain forms of the B7 T cell costimulatory molecules. By analogy to the B7 T cell costimulatory molecule structure, and in view of the similar functional activity for OX-2 (disclosed herein), the existence of naturally-occurring gene(s) encoding novel OX-2 protein T cell costimulatory molecule(s) which contain multiple exons encoding different signal peptide domains (as well as different mRNA transcripts), and encoded proteins is predicted. Thus, the invention embraces isolated nucleic acids which encode proteins which bind to the OX-2 receptor, exhibit a T cell costimulatory activity, and comprise contiguous nucleotide sequences derived from at least one OX-2 T cell costimulatory molecule gene. These nucleotide sequences can be represented by a formula A-B-C-D, wherein

A comprises a nucleotide sequence of at least one first exon of an OX-2 T cell costimulatory molecule gene, wherein the at least one first exon encodes a signal peptide domain,

B comprises a nucleotide sequence of at least one second exon of an OX-2 T cell costimulatory molecule gene, wherein the at least one second exon encodes an immunoglobulin variable region-like domain,

C comprises a nucleotide sequence of at least one third exon of an OX-2 T cell costimulatory molecule gene, wherein the at least one third exon encodes an immunoglobulin constant region-like domain, and

D, which may or may not be present, comprises a nucleotide sequence of at least one fourth exon of an OX-2 T cell costimulatory molecule gene, wherein the at least one fourth exon encodes a transmembrane domain and a cytoplasmic domain, with the proviso that A does not comprise a nucleotide sequence encoding a known OX-2 protein signal peptide domain.

In the formula, A, B, C and D are contiguous nucleotide sequences linked by

phosphodiester bonds in a 5' to 3' orientation from A to D. To produce a soluble form of the OX-2 protein T cell costimulatory molecule, D (which comprises the nucleotide sequence of a transmembrane/cytoplasmic domain), optionally is not present or is replaced by a soluble (hydrophilic) amino acid sequence.

Thus, in one embodiment of the invention, the isolated nucleic acid encodes a protein which (1) binds to the OX-2 protein, (2) is encoded by an OX-2 T cell costimulatory molecule gene having at least one exon encoding a first signal peptide domain and at least one other exon encoding a second signal peptide domain, and (3) exhibits an OX-2 protein T cell costimulatory activity. By analogy to the structure reported for the B7 T cell costimulatory molecules, the at least one exon encoding the first signal peptide domain is a nucleotide sequence encoding the signal peptide domain of the human or rat OX-2 proteins. In this embodiment, the isolated nucleic acid includes a nucleotide sequence encoding at least one second signal peptide domain in addition to or in place of the exon encoding the first signal peptide domain.

B. Nucleic Acids Encoding Proteins With Domains Deleted

Another aspect of the invention pertains to isolated nucleic acids encoding OX-2 protein T cell costimulatory molecules having structural domains which have been deleted. This aspect of the invention is based, at least in part, on the existence of alternatively-spliced forms of B7 mRNA transcripts in which an exon encoding a structural domain has been excised (see, international application PCT/US95/02576).

In one embodiment of the instant invention, the exon encoding the IgV-like domain is deleted and the signal peptide domain exon is linked directly to the IgC-like domain exon to comprise a contiguous nucleotide sequence derived from at least one OX-2 T cell costimulatory molecule gene, the nucleotide sequence represented by a formula A-C-D, wherein

A comprises a nucleotide sequence of at least one first exon of an OX-2 T cell costimulatory molecule gene, wherein the at least one first exon encodes a signal peptide domain,

C comprises a nucleotide sequence of at least one second exon of an OX-2 T cell costimulatory molecule gene, wherein the at least one second exon encodes an immunoglobulin constant region-like domain, and

D comprises a nucleotide sequence of at least one third exon of an OX-2 T cell costimulatory molecule gene, wherein the at least one third exon encodes a transmembrane/cytoplasmic domain.

In the formula, A, C and D are contiguous nucleotide sequences linked by phosphodiester bonds in a 5' to 3' orientation from A to C to D.

Naturally-occurring mRNA transcripts encoding murine B7-1 have been detected in which the exon encoding the IgV-Like domain has been excised and the exon encoding the signal peptide domain is spliced to the exon encoding the IgC-like domain. By analogy to the B7 structure, one embodiment of the present invention embraces the above-described nucleic acid A-C-D. This embodiment encodes an alternatively-spliced form of the OX-2 protein in which the IgV-Like domain exon has been deleted.

In another embodiment, the exon encoding the IgC-like domain is deleted and the IgV-like domain exon is linked directly to the transmembrane/cytoplasmic domain exon. This embodiment encodes a protein comprising a contiguous nucleotide sequence derived from at least one OX-2 T cell costimulatory molecule gene, the nucleotide sequence represented by a formula A-B-D, wherein

A comprises a nucleotide sequence of at least one first exon of an OX-2 T cell costimulatory molecule gene, wherein the at least one first exon encodes a signal peptide domain,

B comprises a nucleotide sequence of at least one second exon of an OX-2 T cell costimulatory molecule gene, wherein the at least one second exon encodes an immunoglobulin variable region-like domain, and

D comprises a nucleotide sequence of at least one third exon of an OX-2 T cell costimulatory molecule gene, wherein the at least one third exon encodes a transmembrane/cytoplasmic domain.

In the formula, A, B and D are contiguous nucleotide sequences linked by phosphodiester bonds in a 5' to 3' orientation from A to B to D.

Isolated nucleic acids encoding alternatively-spliced forms of murine B7-1 in which an IgC-like domain exon has been deleted are described in international application PCT/US95/02576. Naturally-occurring mRNA transcripts encoding murine B7-1 have been detected in which the exon encoding the IgC-like domain has been excised and the exon encoding the IgV-like domain is spliced to the exon encoding the transmembrane domain. When expressed in a host cell, the IgV-like isoform of mB7-1 reportedly was capable of binding to both mouse CTLA4 and mouse CD28 and triggering a costimulatory signal in a T cell. In view of the structural and functional similarities of the OX-2 and B7 costimulatory molecules, alternatively-

spliced, functionally-active forms of the OX-2 protein in which the IgC-like domain exon has been deleted are believed to occur in nature.

II. Isolation of Nucleic Acids of the Invention

An isolated nucleic acid having a nucleotide sequence disclosed herein can be obtained by standard molecular biology techniques. For example, oligonucleotide primers suitable for use in the polymerase chain reaction (PCR) can be prepared based upon the nucleotide sequences disclosed herein and the nucleic acid molecule can be amplified from cDNA and isolated. At least one oligonucleotide primer should be complementary to a nucleotide sequence encoding an alternative structural domain. For example, to identify novel, alternatively-spliced forms of the OX-2 gene, an oligonucleotide primer complementary to a nucleotide sequence encoding the predicted alternative structural domain can be used to screen a cDNA library to isolate and identify a nucleic acid of the invention.

Isolated nucleic acid molecules having nucleotide sequences other than those specifically disclosed herein are also encompassed by the invention. For example, novel structural forms of the OX-2 protein from species other than mouse, rat and human are within the scope of the invention. Furthermore, additional alternatively-spliced forms of the OX-2 protein can be identified using the techniques described herein. These alternatively-spliced forms can be identified and isolated by one of ordinary skill in the art using no more than routine experimentation.

An isolated nucleic acid encoding a novel structural form of an OX-2 protein T cell costimulatory molecule can be obtained by isolating and analyzing cDNA clones encoding the OX-2 protein T cell costimulatory molecule by standard techniques (see, for example, Sambrook et al. Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory press (1989) or other laboratory handbook). For example, cDNAs encoding the OX-2 protein or a functional variant thereof can be amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) using oligonucleotide primers specific for the OX-2 T cell costimulatory molecule gene. The amplified cDNAs then can be subcloned into a plasmid vector and sequenced by standard methods. Oligonucleotide primers for RT-PCR can be designed based upon previously disclosed nucleotide sequences of the OX-2 protein, as well as on the nucleotide sequences disclosed herein. For analyzing the 5' or 3' ends of mRNA transcripts, cDNA can be prepared using a 5' or 3' "RACE" procedure (rapid amplification of cDNA ends) as described in the Examples (see also international application PCT/US95/02576). Alternatively, a cDNA library

can be prepared from a cell line which expresses the OX-2 T cell costimulatory molecule by screening the library with a probe containing all or a portion of the nucleotide sequence encoding the OX-2 T cell costimulatory molecule. The screening procedure is performed under highly stringent conditions in order to selectively identify those sequences having substantial sequence homology to the nucleic acid encoding the OX-2 protein, and in particular, to the nucleic acid encoding the IgV-like and/or IgC-like domains.

Individual isolated cDNA clones encoding an OX-2 T cell costimulatory molecule then can be sequenced by standard techniques, such as dideoxy sequencing or Maxam-Gilbert sequencing, to identify a cDNA clone encoding an OX-2 T cell costimulatory molecule having a novel structural domain. A novel structural domain can be identified by comparing the sequence of the cDNA clone to the previously disclosed nucleotide sequences encoding OX-2 T cell costimulatory molecules (SEQ ID NOS: 1 and 3). Once an alternative structural domain has been identified, the nucleotide sequence encoding the domain can be mapped in genomic DNA to determine whether the domain is encoded by a novel exon. This type of approach provides the most extensive information about alternatively-spliced forms of mRNAs encoding the OX-2 costimulatory molecules.

Alternatively, a novel structural domain for an OX-2 T cell costimulatory molecule can be identified in genomic DNA by identifying a novel exon in the gene encoding the OX-2 T cell costimulatory molecule. A novel exon can be identified as an open reading frame flanked by splice acceptor and splice donor sequences. Genomic clones encoding an OX-2 T cell costimulatory molecule can be isolated by screening a genomic DNA library with a probe encompassing all or a portion of a nucleotide sequence encoding the OX-2 T cell costimulatory molecule (e.g., having all or a portion of a nucleotide sequence shown in SEQ ID NOS: 1, 3 or 5). For costimulatory molecules derived from genes that have been mapped to a particular chromosome, a chromosome-specific library rather than a total genomic DNA library can be used. For example, the OX-2 gene has been mapped to human chromosome 3. Genomic clones can be sequenced by conventional techniques and novel exons identified. A probe corresponding to a novel exon then can be used to detect the nucleotide sequence of this exon in mRNA transcripts encoding the OX-2 T cell costimulatory molecule (e.g., by screening a cDNA library or by PCR).

A more preferred approach for identifying and isolating nucleic acid encoding novel structural domains is by "exon trapping." Exon trapping is a technique that has been used

successfully to identify and isolate novel exons (see e.g. Duyk, G.M. et al. (1990) Proc. Natl. Acad. Sci. USA 21:8995-8999; Auch, D. and Reth, M. (1990) Nucleic Acids Res. 11:6743-6744; Hamaguchi, M. et al. (1992) Proc. Natl. Acad. Sci. USA 12:9779-9783; and Krizman, D.B and Berget, S.M. (1993) Nucleic Acids Res. 21:5198-5202). The approach of exon trapping can be applied to identify and isolate exons encoding novel structural domains of OX-2 T cell costimulatory molecules.

In addition to the isolated nucleic acids encoding naturally-occurring alternatively-spliced forms of the OX-2 T cell costimulatory molecules, it will be appreciated by those skilled in the art that nucleic acids encoding alternative variant forms, which may or may not occur naturally, can be obtained using standard recombinant DNA techniques. The terms "alternative variant forms" or "variants" as used herein refer to novel combinations of exon sequences which can be created using recombinant DNA techniques. That is, novel or known exons encoding structural domains of OX-2 T cell costimulatory molecules, either provided by the invention or identified according to the teachings of the invention, can be "spliced", using standard recombinant DNA techniques, to other exons encoding other structural domains of the OX-2 costimulatory molecule, regardless of whether the particular combination of exons has been observed in nature. Thus, novel combinations of exons can be linked in vitro to create alternative variant forms of nucleic acids encoding novel OX-2 T cell costimulatory molecules. For example, a structural form of murine B7-1 which has a signal peptide domain directly joined to the IgC-like domain (i.e., which has the IgV-like domain deleted) has been observed in nature in combination with the cytoplasmic domain encoded by the B7-1 exon 5. Using conventional techniques, analogous structural forms of the OX-2 nucleic acid can be created in which the IgV-like domain is deleted and the OX-2 signal peptide domain is directly joined to the IgC-like domain. Additionally, an exon encoding a structural domain of one OX-2 T cell costimulatory molecule can be transferred to another T cell costimulatory molecule by standard techniques.

For the purposes of this invention, the amino acid residues encompassing the different "domains" (i.e., signal (S), IgV-like (V), IgC-like (C), transmembrane/cytoplasmic (TM/Cyt)) of human, rat and mouse OX-2 proteins are defined as follows: rat OX-2 (as shown in SEQ ID NO: 4): (-30)-(-1) (S), (1)-(111) (V), (112)-(203) (C), (203)-(248) (TM/Cyt). The amino acid numbering is based upon that used by Clark et al., EMBO 4(1):113-118, 1985, in reference to rat OX-2. The rat, human and mouse OX-2 have the identical structural organization. It will be appreciated by the skilled artisan that regions slightly longer or shorter than these amino acid

domains (i.e., a few amino acid residues more or less at either the amino-terminal or carboxy-terminal end) may be equally suitable for use as signal, IgV-like, IgC-like and/or transmembrane/cytoplasmic domains in the proteins of the invention (i.e., there is some flexibility in the junctions between different domains within the proteins of the invention as compared to the domain junctions delineated above for OX-2 proteins). Accordingly, proteins comprising signal, IgV-like, IgC-like and/or transmembrane/cytoplasmic domains having essentially the same amino acid sequences as those regions delineated above but which differ from the above-delineated junctions merely by a few amino acid residues, either longer or shorter, at either the amino- or carboxy-terminal end of the domain are intended to be encompassed by the invention. Nucleic acid segments encoding any of the domains delineated above can be obtained by standard techniques, e.g., by PCR amplification using oligonucleotide primers based on the nucleotide sequences disclosed herein, and can be ligated together to create nucleic acid molecules encoding recombinant forms of the proteins of the invention.

It will also be appreciated by those skilled in the art that changes can be made in the nucleotide sequences provided by the invention without changing the encoded protein due to the degeneracy of the genetic code. Additionally, nucleic acids which have a nucleotide sequence different from those disclosed herein due to degeneracy of the genetic code may be isolated from biological sources. Such nucleic acids encode functionally equivalent proteins (e.g., a protein having an OX-2 T cell costimulatory activity as described above) to those described herein. For example, a number of amino acids are designated by more than one triplet codon. Codons that specify the same amino acid, or synonyms (for example, CAU and CAC are synonyms for histidine) may occur in isolated nucleic acids from different biological sources or can be introduced into an isolated nucleic acid by standard recombinant DNA techniques without changing the protein encoded by the nucleic acid. Isolated nucleic acids encoding alternatively-spliced forms of OX-2 T cell costimulatory molecules having a nucleotide sequence which differs from those provided herein due to the degeneracy of the genetic code are considered to be within the scope of the invention. The invention also embraces nucleic acids which encode functionally equivalent protein variants (OX-2 functional variants) in which the variant differs from the naturally-occurring OX-2 protein by including conservative amino acid substitutions. As used herein, "conservative amino acid substitution" refers to an amino acid substitution which does not alter the relative charge or size characteristics of the peptide in which the amino acid substitution is made. Conservative substitutions of amino acids include substitutions made

amongst amino acids within the following groups: (a) MILV; (b) FYW; (c) KRH; (d) AG; (e) ST; (f) QN; and (g) ED.

III. Additional Isolated Nucleic Acid Molecules of the Invention

In addition to isolated nucleic acids encoding alternative forms of OX-2 protein T cell costimulatory molecules, the invention also discloses the previously undescribed nucleotide sequences of the murine OX-2 gene and the encoded OX-2 protein (see SEQ ID NOS: 5 and 6).

IV. Uses for the Isolated Nucleic Acid Molecules of the Invention

A. Probes

The isolated nucleic acids of the invention are useful for constructing nucleotide probes for detecting nucleotide sequences in biological materials, such as cell extracts, or directly in cells (e.g., by in situ hybridization). A nucleotide probe can be labeled with a radioactive element which provides for an adequate signal as a means for detection and has sufficient half-life to be useful for detection, such as ^{32}P, ^{3}H, ^{14}C or the like. Other materials which can be used to label the probe include antigens that are recognized by a specific labeled antibody, fluorescent compounds, enzymes and chemiluminescent compounds. An appropriate label can be selected with regard to the rate of hybridization and binding of the probe to the nucleotide sequence to be detected and the amount of nucleotide available for hybridization. The isolated nucleic acids of the invention, or unique fragments thereof, can be used as suitable probes for a variety of hybridization procedures well known to those skilled in the art. The isolated nucleic acids of the invention enable one to determine whether a cell expresses an alternatively-spliced form of an OX-2 T cell costimulatory molecule. For example, mRNA can be prepared from a sample of cells to be examined and the mRNA can be hybridized to an isolated nucleic acid encompassing a nucleotide sequence encoding all or a portion of an alternative cytoplasmic domain of an OX-2 protein T cell costimulatory molecule to detect the expression of the alternative cytoplasmic domain form of the OX-2 costimulatory molecule in the cells. Furthermore, the isolated nucleic acids of the invention can be used to design oligonucleotide primers, e.g. PCR primers, which allow one to detect the expression of an alternatively-spliced form of an OX-2 T cell costimulatory molecule. Preferably, this oligonucleotide primer spans a novel exon junction created by alternative splicing and thus can only amplify cDNAs encoding this alternatively-spliced form. For example, an oligonucleotide primer which spans exon 4 and exon 6 of murine B7-1 reportedly can be used to distinguish between the expression of a first cytoplasmic domain form of mB7-1 and expression of an alternative second cytoplasmic domain form of the B7-1

costimulatory molecule (e.g., see international application PCT/US95/02576, Example 2). Novel, alternatively-spliced forms of the OX-2 T cell costimulatory molecule can be detected by designing and using PCR primers in an analogous manner to that described in international application PCT/US95/02576 in reference to discovering novel, alternatively-spliced forms of the B7 T cell costimulatory molecules.

The probes of the invention can be used to detect an alteration in the expression of an alternatively-spliced form of an OX-2 T cell costimulatory molecule which may occur, for example, in a disease state. For example, detection of a defect in the expression of an alternatively-spliced form of an OX-2 T cell costimulatory molecule that is associated with an immunodeficiency disorder can be used to diagnose the disorder (i.e., the probes of the invention can be used for diagnostic purposes) and to identify conditions that are treatable by administration of an OX-2 therapeutic agent. Many congenital immunodeficiency diseases result from lack of expression of a cell-surface antigen that is important for interactions between T cells and antigen presenting cells. For example, the bare lymphocyte syndrome results from lack of expression of MHC class II antigens (see e.g., Rijkers, G.T. et al. (1987) J Clin. Immunol. 7:98-106; Hume, C.R. et al. (1989) Hum. Immunol. 25:1-11)) and X-linked hyperglobulinemia results from defective expression of the ligand for CD40 (gp39) (see e.g. Korthauer, U et al. (1993) Nature 361:541; Aruffo, A. et al. (1993) Cell 72:291-300). An immunodeficiency disorder which results from the lack (or significantly reduced) expression of OX-2 or of an alternatively-spliced form of OX-2 can be diagnosed using a probe of the invention. For example, a disorder resulting from the lack or reduced expression of the OX-2 protein can be diagnosed in a patient based upon the inability of a probe which detects this OX-2 nucleic acid to hybridize to mRNA in cells from the patient (e.g., by RT-PCR or by Northern blotting). The probes of the invention also can be used for in vivo imaging.

B. Recombinant Expression Vectors

An isolated nucleic acid of the invention can be incorporated into an expression vector (i.e., a recombinant expression vector) to direct expression of a novel structural form of an OX-2 T cell costimulatory molecule encoded by the nucleic acid. The recombinant expression vectors are suitable for transformation of a host cell, and include a nucleic acid (or fragment thereof) of the invention and a regulatory sequence, selected on the basis of the host cells to be used for expression, which is operatively-linked to the nucleic acid. Operatively linked is intended to mean that the nucleic acid is linked to a regulatory sequence in a manner which allows

expression of the nucleic acid. Regulatory sequences are art-recognized and are selected to direct expression of the desired protein in an appropriate host cell. Accordingly, the term regulatory sequence includes promoters, enhancers and other expression control elements. Such regulatory sequences are known to those skilled in the art or are described in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). It should be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transfected and/or the type of protein desired to be expressed. Such expression vectors can be used to transfect cells to thereby produce proteins or peptides encoded by nucleic acids as described herein.

The recombinant expression vectors of the invention can be designed for expression of encoded proteins in prokaryotic or eukaryotic cells. For example, proteins can be expressed in bacterial cells such as E coli, insect cells (using baculovirus), yeast cells or mammalian cells. Other suitable host cells can be found in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). Expression in prokaryotes is most often carried out in E. coli with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids usually to the amino terminus of the expressed target gene. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the target recombinant protein; and 3) to aid in the purification of the target recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the target recombinant protein to enable separation of the target recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Amrad Corp., Melbourne, Australia), pMAL (New England Biolabs, Beverly, NM) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase, maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Inducible non-fusion prokaryotic expression vectors include pTrc (Amann et al., (1988) Gene 62:301-315) and pET11d (Studier et al., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, California (1990) 60-89). In pTrc, target gene expression relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. In pET11d, expression of inserted target genes relies on transcription from the T7 gn10-lac 0

fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gnl). This viral polymerase is supplied by host strains BL21 (DE3) or HMS 174(DE3) from a resident prophage harboring a T7 gn 1 under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant protein expression in E. coli is to express the protein in a host bacterial strain with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., Gene Expression Technology: Methods in Enzymology 185 Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector (e.g., a nucleic acid of the invention) so that the individual codons for each amino acid would be those preferentially utilized in highly expressed E coli proteins (Wada et al., (1992) Nuc. Acids Res. 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques and are encompassed by the invention.

Examples of vectors for expression in yeast S. cerivisae include pYepSec 1 (Baldari, et al., (1987) EMBO J. 6:229-234), pMFa (Kurjan and Herskowitz, (1982) Cell 30:933-943), pJRY88 (Schultz et al., (1987) Gene 54:113-123), and pYES2 (Invitrogen Corporation, San Diego, CA). Baculovirus vectors available for expression of proteins in cultured insect cells (SF 9 cells) include the pAc series (Smith et al., (1983) Mol Cell Biol 2:2156-2165) and the pVL series (Lucklow, V.A., and Summers, M.D., (1989) Virology 170:31-39).

Expression of alternatively-spliced forms of OX-2 T cell costimulatory molecules in mammalian cells is accomplished using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B., (1987) Nature 329:840) and pMT2PC (Kaufman et al. (1987), EMBO J 6:187-195, U.S. Patent No. 5,449,614, issued to Danos et al.). When used in mammalian cells, the control functions of the expression vectors often are provided by viral material. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. The recombinant expression vector can be designed such that expression of the nucleic acid occurs preferentially in a particular cell type. In this situation, the expression vector's control functions are provided by regulatory sequences which allow for preferential expression of a nucleic acid contained in the vector in a particular cell type, thereby allowing for tissue or cell specific expression of an encoded protein.

The recombinant expression vectors of the invention can be a plasmid or virus, or viral portion which allows for expression of a nucleic acid introduced into the viral nucleic acid. For example, replication defective retroviruses, adenoviruses and adeno-associated viruses can be

used. The recombinant expression vectors can be introduced into a host cell, e.g. in vitro or in vivo. A host cell line can be used to express a protein of the invention. Furthermore, introduction of a recombinant expression vector of the invention into a host cell can be used for therapeutic purposes when the host cell is defective in expressing the novel structural form of the OX-2 T cell costimulatory molecule. For example, a recombinant expression vector of the invention can be used for gene therapy purposes in a patient with an immunodeficiency disorder which results from inadequate expression of OX-2 or of a novel structural form of the OX-2 T cell costimulatory molecule. See, e.g., U.S. Patent No. 5,399,346, issued to Anderson et al., and PCT application No. PCT/US94/06809 (WO95/00654), for exemplary gene therapy protocols and related compositions.

C. Host Cells

The invention further provides a host cell transfected with a recombinant expression vector of the invention. The term "host cell" is intended to include prokaryotic and eukaryotic cells into which a recombinant expression vector of the invention can be introduced. The terms "transformed with", "transfected with", "transformation" and "transfection" are intended to encompass the introduction of nucleic acid (e.g., a vector) into a cell by one of a number of possible techniques known in the art. Prokaryotic cells can be transformed with nucleic acid by, for example, electroporation or calcium-chloride mediated transformation. Nucleic acid can be introduced into mammalian cells via conventional techniques such as calcium phosphate co-precipitation, DEAE-dextram-mediated transfection, lipofectin, electroporation or microinjection. Suitable methods for transforming and transfecting host cells can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory press (1989)), and other laboratory handbooks.

The number of host cells transfected with a recombinant expression vector of the invention by techniques such as those described above will depend upon the type of recombinant expression vector used and the type of transfection technique used. Typically, plasmid vectors introduced into mammalian cells are integrated into host cell DNA at only a low frequency. In order to identify these integrants, a gene that contains a selectable marker (i.e., resistance to antibiotics) can be introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to certain drugs, such as geneticin (G418) and hygromycin. Selectable markers can be introduced on a separate vector (e.g., plasmid) from the nucleic acid of interest or, preferably, are introduced on the same vector (e.g.,

plasmid). Host cells transformed with one or more recombinant expression vectors containing a nucleic acid of the invention and a gene for a selectable marker can be identified by selecting for cells using the selectable marker. For example, if the selectable marker encoded a gene conferring neomycin resistance, transformant cells can be selected with G418. Cells that have incorporated the selectable marker gene will survive, while the other cells die.

In another embodiment, the host cell transfected with a recombinant expression vector encoding a novel structural form of an OX-2 T cell costimulatory molecule is a tumor cell. Expression of the Cyt-I form of murine B7-1 on the surface of B7-1 negative murine tumor cells has been shown to induce T cell mediated specific immunity against the tumor cells. The induced immunity was accompanied by tumor rejection and prolonged protection to tumor challenge in mice (see Chen, L., et al. (1992) Cell 71, 1093 -1102; Townsend, S.E. and Allison, J.P. (1993) Science 259:368-370; Baskar, S., et al. (1993) Proc. Natl. Acad. Sci. 90:5687-5690). Similarly, expression of functionally-active, novel structural forms of OX-2 T cell costimulatory molecules ("OX-2 functional variants") on the surface of a tumor cell should be useful for increasing the immunogenicity of the tumor cell. For example, tumor cells obtained from a patient can be transfected ex vivo with a recombinant expression vector of the invention, e.g., encoding an alternative cytoplasmic domain form of an OX-2 costimulatory molecule, and the transfected tumor cells then can be returned to the patient. Alternatively, gene therapy techniques can be used to target a tumor cell for transfection in vivo. Additionally, the tumor cell can also be transfected with recombinant expression vectors encoding other proteins to be expressed on the tumor cell surface to increase the immunogenicity of the tumor cell. International application PCT/US95/02576 describes the expression of the Cyt-I form of B7-1, B7-2, MHC molecules (e.g., class I and/or class II) and/or adhesion molecules on tumor cells in conjunction with the Cyt-II form of B7-1 to enhance an immune response to tumor cell antigens. In an analogous manner, the naturally-occurring form(s) of OX-2, OX-2 agonists, MHC molecules, adhesion molecules and/or cytokines can be expressed on the tumor cells to enhance an immune response to tumor cell antigens.

D. Anti-Sense Nucleic Acid Molecules

The isolated nucleic acid molecules of the invention can also be used to design antisense nucleic acid molecules, or oligonucleotide fragments thereof, that can be used to modulate the expression of OX-2, as well as alternative, naturally-occurring forms of the OX-2 T cell costimulatory molecule. An antisense nucleic acid comprises a nucleotide sequence which is

complementary to a coding strand of a nucleic acid, e.g. complementary to an mRNA sequence according to the rules of Watson and Crick base pairing, and can hydrogen bond to the coding strand of a target nucleic acid (e.g., the OX-2 mRNA). The hydrogen bonding of an antisense nucleic acid molecule to an mRNA transcript can prevent translation of the mRNA transcript and thus inhibit the production of the protein encoded therein. Accordingly, an antisense nucleic acid molecule can be designed which is complementary to a nucleotide sequence encoding a novel structural domain of an OX-2 T cell costimulatory molecule to inhibit production of that particular structural form of the OX-2 T cell costimulatory molecule.

For applications directed to the use of an isolated oligonucleotide as an antisense nucleic acid for regulating transcription and/or translation of, for example, the extracellular domain of OX-2, the preferred oligonucleotide is an antisense oligonucleotide between about 10 and about 100 nucleotides in length. The antisense oligonucleotide is capable of hybridizing under high stringency conditions to unique fragments of the extracellular domain of SEQ ID NO:1. As used herein, "antisense oligonucleotide" refers to an oligonucleotide (DNA, RNA and/or oligonucleotides containing non-naturally-occurring nucleotides which retain the ability to base pair with a nucleic acid target) that is capable of hybridizing to the naturally-occurring DNA or mRNA encoding the known OX-2 protein or naturally-occurring structural and functional variants, thereof. In a preferred embodiment, the antisense oligonucleotide is capable of hybridizing in vivo to a unique portion of the nucleic acid encoding the signal sequence, the IgV-like and/or IgC-like domains, or the transcription products of the nucleic acid encoding these domains. Base-pairing of the antisense oligonucleotide with the DNA (or RNA) encoding the signal sequence or the extracellular domains of OX-2 in vivo, prevents costimulation of T cells by preventing transcription (or translation) of OX-2 in vivo.

An antisense nucleic acid molecule, or oligonucleotide fragment thereof, can be constructed by chemical synthesis and enzymatic ligation reactions using procedures known in the art. The antisense nucleic acid or oligonucleotide can be chemically synthesized using naturally-occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Alternatively, the anti-sense nucleic acids and oligonucleotides can be produced biologically using an expression vector into which a nucleic

acid has been subcloned in an antisense orientation (i.e. nucleic acid transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest). The antisense expression vector is introduced into cells in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using anti-sense genes see Weintraub, H. et al., "Antisense RNA as a molecular tool for genetic analysis", Reviews - Trends in Genetics, Vol. 1(1) 1986.

F. Non-Human Transgenic and Homologous Recombinant Animals

The isolated nucleic acids of the invention can further be used to create a non-human transgenic animal. A transgenic animal is an animal having cells that contain a transgene, wherein the transgene was introduced into the animal or an ancestor of the animal at a prenatal, e.g., an embryonic, stage. A transgene is a DNA molecule which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. Accordingly, the invention provides a non-human transgenic animal which contains cells transfected to express an alternative form of an OX-2 T cell costimulatory molecule. Preferably, the non-human animal is a mouse. A transgenic animal can be created, for example, by introducing a nucleic acid encoding the protein (typically linked to appropriate regulatory elements, such as a tissue-specific enhancer) into the male pronuclei of a fertilized oocyte, e.g., by microinjection, and allowing the oocyte to develop in a pseudopregnant female foster animal. For example, a transgenic animal (e.g., a mouse) which expresses an OX-2 protein can be made using the isolated nucleic acid shown in SEQ ID NOs:3 or 5, or the isolated hOX-2 nucleic acid represented in SEQ ID Nos:1, 11, 12 and 19. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. These isolated nucleic acids can be linked to regulatory sequences which direct the expression of the encoded protein in one or more particular cell types. Methods for generating transgenic animals, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009 and Hogan, B. et al., (1986) A Laboratory Manual, Cold Spring Harbor, New York, Cold Spring Harbor Laboratory. A transgenic founder animal can be used to breed additional animals carrying the transgene.

The isolated nucleic acids of the invention can be used to create a non-human homologous recombinant animal. The term "homologous recombinant animal" as used herein is intended to describe an animal containing a gene which has been modified by homologous recombination. The homologous recombination event may completely disrupt the gene such that a functional gene product can no longer be produced (often referred to as a "knock-out" animal) or the homologous recombination event may modify the gene such that an altered, although still functional, gene product is produced. Preferably, the non-human animal is a mouse. For example, an isolated nucleic acid of the invention can be used to create a homologous recombinant mouse in which a recombination event has occurred in the OX-2 gene at an exon encoding a cytoplasmic domain such that this exon is altered. Accordingly, the invention provides a non-human knock-out animal which contains a gene encoding an OX-2 protein wherein, for example, an exon encoding a naturally-occurring known or a novel cytoplasmic domain is disrupted or altered.

To create an animal with homologously recombined nucleic acid, a vector is prepared which contains the DNA sequences which are to replace the endogenous DNA sequences, flanked by DNA sequences homologous to flanking endogenous DNA sequences (see for example Thomas, K.R. and Capecchi, M. R. (1987) Cell 51:503). The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced DNA has homologously recombined with the endogenous DNA are selected (see for example Li, E. et al. (1992) Cell 69:915). The selected cells are then injected into a blastocyst of an animal (e.g., a mouse) to form aggregation chimeras (see for example Bradley, A. in Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo then can be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA.

V. Isolated Novel Forms of OX-2 Protein Costimulatory Molecules

The invention further provides isolated OX-2 T cell costimulatory molecules encoded by the nucleic acids of the invention. These molecules have a novel structural form, either containing a novel structural domain or having a structural domain deleted. As used herein the term "isolated," in reference to a protein, refers to an OX-2 T cell costimulatory molecule that is substantially free of cellular material or culture medium when produced by recombinant DNA

techniques, or chemical precursors or other chemicals when chemically synthesized.

A. Proteins with a Novel Signal Peptide Domain

In one aspect of the invention, OX-2 T cell costimulatory molecules which include at least one novel signal peptide domain are provided. In one embodiment, the isolated OX-2 T cell costimulatory molecule binds to the OX-2 receptor and has an amino acid sequence derived from the amino acid sequences encoded by at least one OX-2 T cell costimulatory molecule gene. In this embodiment, the OX-2 T cell costimulatory molecule comprises a contiguous amino acid sequence represented by a formula A-B-C-D, wherein

A comprises an amino acid sequence of a signal peptide domain encoded by at least one exon of an OX-2 T cell costimulatory molecule gene,

B comprises an amino acid sequence of an immunoglobulin variable region-like domain encoded by at least one exon of an OX-2 T cell costimulatory molecule gene,

C comprises an amino acid sequence of an immunoglobulin constant region-like domain encoded by at least one exon of an OX-2 T cell costimulatory molecule gene,

D, which may or may not be present, comprises an amino acid sequence of a transmembrane/cytoplasmic domain encoded by at least one exon of an OX-2 T cell costimulatory molecule gene

with the proviso that A does not comprise an amino acid sequence of an OX-2 signal peptide domain of the prior art.

In the formula, A, B, C, and D are contiguous amino acid residues linked by amide bonds from an N-terminus to a C-terminus. In one embodiment, a soluble form of the OX-2 T cell costimulatory molecule comprises the above-described molecule with the exception that D (the transmembrane/cytoplasmic domain) is absent.

B. Isolated Proteins with Structural Domains Deleted

This invention also embraces costimulatory molecules which have at least one structural domain deleted. In one embodiment, the structural form has at least one IgV-like domain deleted. Accordingly, in one embodiment, the isolated protein has an amino acid sequence derived from amino acid sequences encoded by at least one OX-2 T cell costimulatory molecule gene and comprises a contiguous amino acid sequence represented by a formula A-C-D, wherein

A, which may or may not be present, comprises an amino acid sequence of

a signal peptide domain encoded by at least one exon of an OX-2 T cell costimulatory molecule gene.

C comprises an amino acid sequence of an immunoglobulin constant region-like domain encoded by at least one exon of an OX-2 T cell costimulatory molecule gene, and

D comprises an amino acid sequence of a transmembrane/cytoplasmic domain encoded by at least one exon of an OX-2 T cell costimulatory molecule gene.

In the formula, A, C and D are contiguous amino acid residues linked by amide bonds from an N-terminus to a C-terminus.

In another embodiment, the structural form of the OX-2 T cell costimulatory molecule has at least one IgC-like domain deleted. Accordingly, in this embodiment, the isolated protein has an amino acid sequence derived from amino acid sequences encoded by at least one OX-2 T cell costimulatory molecule gene and comprises a contiguous amino acid sequence represented by a formula A-B-D, wherein

A, which may or may not be present, comprises an amino acid sequence of a signal peptide domain encoded by at least one exon of an OX-2 T cell costimulatory molecule gene,

B comprises an amino acid sequence of an immunoglobulin variable region-like domain encoded by at least one exon of a T cell costimulatory molecule gene, and

D comprises an amino acid sequence of a transmembrane/cytoplasmic domain encoded by at least one exon of an OX-2 T cell costimulatory molecule gene.

In the formula, A, B and D are contiguous amino acid residues linked by amide bonds from an N-terminus to a C-terminus.

The proteins of the invention can be isolated by expression of the molecules (e.g., proteins or peptide fragments thereof) in a suitable host cell using techniques known in the art. Suitable host cells include prokaryotic or eukaryotic organisms or cell lines, for example, yeast, E. coli and insect cells. The recombinant expression vectors of the invention, described above, can be used to express a costimulatory molecule in a host cell in order to isolate the protein. The invention provides a method of preparing an isolated protein of the invention comprising

introducing into a host cell a recombinant expression vector encoding the protein, allowing the protein to be expressed in the host cell and isolating the protein. Proteins can be isolated from a host cell expressing the protein according to standard procedures of the art, including ammonium sulfate precipitation, fractionation, column chromatography (e.g. ion exchange, gel filtration, electrophoresis, affinity chromatography, etc.) and ultimately, crystallization (see generally, 'Enzyme Purification and Related Techniques', Methods in Enzymology, 22:233-577 (1971)).

Alternatively, the costimulatory molecules of the invention can be prepared by chemical synthesis using techniques well known in the chemistry of proteins such as solid phase synthesis (Merrifield, 1964, J. Am. Chem. Assoc. 85:2149-2154) or synthesis in homogeneous solution (Houbenweyl, 1987, Methods of Organic Chemistry, ed. E. Wansch, Vol. 15 I and II, Thieme, Stuttgart).

VI. Uses For the Novel T Cell Costimulatory Molecules of the Invention

A. Costimulation

The OX-2 T cell costimulatory molecules of the invention can be used to trigger a costimulatory signal in T cells. When membrane-bound or in a multivalent form, an OX-2 T cell costimulatory molecule can trigger a costimulatory signal in a T cell by allowing the costimulatory molecule to interact with its receptor on T cells (the OX-2 receptor) in the presence of a primary activation signal. An OX-2 T cell costimulatory molecule of the invention can be obtained in membrane-bound form by expressing the molecule in a host cell (e.g., by transfecting the host cell with a recombinant expression vector encoding the molecule). To be expressed on the surface of a host cell, the OX-2 T cell costimulatory molecule should include extracellular domains (i.e., signal peptide, which may or may not be present in the mature protein, IgV-like and IgC-like domains), and a transmembrane/cytoplasmic domain. To trigger a costimulatory signal, T cells are contacted with the cells expressing the OX-2 costimulatory molecules, preferably together with a primary activation signal (e.g., MHC-associated antigenic peptide, anti-CD3 antibody, phorbol ester etc.). Activation of the T cell can be assayed by standard procedures, for example, by measuring T cell proliferation or cytokine production. For applications directed to the use of an isolated oligonucleotide for regulating transcription and/or translation of the extracellular domain of OX-2, the preferred oligonucleotide is an antisense oligonucleotide between about 10 and about 100 nucleotides in length. The antisense oligonucleotide is capable of hybridizing under high stringency conditions to unique fragments of the extracellular domains of SEQ ID NO: 1. As used herein, "antisense oligonucleotide"

refers to an oligonucleotide (DNA and/or RNA) that is capable of hybridizing to the naturally-occurring DNA or mRNA encoding the human OX-2 under stringent conditions (e.g., antisense molecules which specifically hybridize to the OX-2 signal sequence of extracellular domains). In a preferred embodiment, the antisense oligonucleotide is capable of hybridizing in vivo to the nucleotide sequence encoding amino acids -19 to -13 (Clark et al., ibid., numbering scheme) of the OX-2 protein or its transcription product. Base-pairing of the antisense oligonucleotide with the DNA (or RNA) encoding the signal sequence or extracellular domains of OX-2 in vivo, prevents costimulation by preventing transcription (or translation) of OX-2. Example 1 describes a screening assay for identifying OX-2 agonists and OX-2 antagonists that is based upon the ability of these agents to costimulate (or inhibit costimulation of) (1) $CD4^+$ T cells and/or (2) thymocytes in an antigen dependent context.

The OX-2 T cell costimulatory molecules of the invention can also be used to inhibit or block a costimulatory signal in T cells. A soluble form of an OX-2 T cell costimulatory molecule which binds to the OX-2 receptor but which does not exhibit a T cell costimulatory activity can be used to competitively inhibit the interaction of membrane-bound costimulatory molecules with the OX-2 receptor on T cells. Such soluble OX-2 antagonists can be identified in the above-noted screening assays. A soluble form of an OX-2 T cell costimulatory molecule can be expressed in a host cell line such that it is secreted by the host cell line and can be purified. Alternatively, the soluble costimulatory molecule contains extracellular domains (signal peptide, which may or may not be present in the mature protein, IgV-like and IgC-like domains) but does not contain a transmembrane/cytoplasmic domain. The soluble form of the OX-2 T cell costimulatory molecule can be in the form of a fusion protein, e.g. an immunoglobulin fusion protein wherein the extracellular portion of the costimulatory molecule is fused to an immunoglobulin constant region. Soluble forms of the OX-2 protein can be evaluated for T cell costimulatory activity in the above-noted screening assays to identify soluble OX-2 agonists and soluble OX-2 antagonists for use in accordance with the methods of the invention. Thus, for example, a soluble form of an OX-2 T cell costimulatory molecule (an OX-2 antagonist) can be used to inhibit a costimulatory signal in T cells by contacting the T cells with the soluble molecule and allowing the antagonist to competitively inhibit binding of the in vivo generated OX-2 protein to its receptor in vivo.

B. Antibodies

The OX-2 T cell costimulatory molecules of the invention can be used to produce

antibodies directed against the costimulatory molecule, and, in particular, to produce antibodies which specifically interact with particular domains of the OX-2 T cell costimulatory molecule to inhibit OX-2 T cell costimulatory activity. Conventional methods can be used to prepare the antibodies. For example, to produce polyclonal antibodies, a mammal, (e.g., a mouse, hamster, or rabbit) can be immunized with a costimulatory molecule, or an immunogenic portion thereof, which elicits an antibody response in the mammal. Techniques for conferring immunogenicity on a protein include conjugation to carriers or other techniques well known in the art. For example, the protein can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassay can be used with the immunogen as antigen to assess the levels of antibodies. Following immunization, antisera can be obtained and, if desired, polyclonal antibodies isolated from the serum.

In addition to polyclonal antisera, the OX-2 costimulatory molecules of the invention can be used to raise monoclonal antibodies. To produce monoclonal antibodies, antibody producing cells (lymphocytes) can be harvested from an immunized animal and fused with myeloma cells by standard somatic cell fusion procedures, thus immortalizing these cells and yielding hybridoma cells. Such techniques are well known in the art. For example, the hybridoma technique originally developed by Kohler and Milstein (Nature 256:495-497 (1975)) as well as other techniques such as the human B-cell hybridoma technique (Kozbor et al., Immunol. Today 4, 72 (1983)), the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., Monoclonal Antibodies in Cancer Therapy (1985) Allen R. Bliss, Inc., pages 77-96), and screening of combinatorial antibody libraries (Huse et al., Science 246: 1275 (1989)). Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with the protein or portion thereof and monoclonal antibodies isolated.

The term antibody as used herein is intended to include fragments thereof which are also specifically reactive with the OX-2 costimulatory molecule. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example, $F(ab')_2$ fragments can be generated by treating the antibody with pepsin. The resulting $F(ab')_2$ fragment can be treated to reduce disulfide bridges to produce Fab' fragments.

Chimeric and humanized antibodies also are within the scope of the invention. It is expected that chimeric and humanized antibodies would be less immunogenic in a human subject

than the corresponding non-chimeric antibodies. A variety of approaches for making chimeric antibodies, comprising for example a non-human variable region and a human constant region, have been described. See, for example, Morrison et al., Proc. Natl. Acad. Sci. USA 81:6851 (1985); Takeda et al., Nature 314, 452 (1985), Cabilly et al., U.S. Patent No. 4,816,567; Boss et al., U.S. Patent No. 4,816,397; Tanaguchi et al., European Patent Publication EP 171496; European Patent Publication 0173494, United Kingdom Patent GB 2177096B. Additionally, a chimeric antibody can be further "humanized" such that parts of the variable regions, especially the conserved framework regions of the antigen-binding domain, are of human origin and only the hypervariable regions are of non-human origin. Such altered immunoglobulin molecules may be made by any of several techniques known in the art, (e.g., Teng et al., Proc. Natl. Acad. Sci. USA, 80:7308-7312 (1983); Kozbor et al., Immunology Today, 4:7279 (1983); Olsson et al., Meth. Enzymol. 92, 3-16 (1982)), and are preferably made according to the teachings of PCT Publication WO92/06193 or EP 0239400. Humanized antibodies can be commercially produced by, for example, Scotgen Limited, 2 Holly Road, Twickenham, Middlesex, Great Britain.

Another method of generating specific antibodies, or antibody fragments, reactive against a novel OX-2 protein or nucleic acid of the invention is to screen phage expression libraries encoding immunoglobulin genes, or portions thereof, with proteins produced from the nucleic acid molecules of the present invention or with the nucleic acids themselves. For example, complete Fab fragments, Vh regions and Fv regions can be expressed in bacteria using phage expression libraries. See for example Ward et al., Nature 341:544-546: (1989); Huse et al., Science 246:1275-1281 (1989); and McCafferty et al., Nature 348:552-554 (1990).

In a preferred embodiment, the invention provides an antibody which specifically binds to a novel structural domain of an OX-2 T cell costimulatory molecule provided by the invention or to the nucleic acid encoding the novel structural domain. The preferred antibodies of the invention do not cross-react with other members of the immunoglobulin superfamily, such as the B7 T cell costimulatory molecules. Such antibodies, and uses therefor, are described in greater detail below in subsection VI, part B.

C. Screening Assays

An OX-2 T cell costimulatory molecule of the invention can be used in a screening assay to identify components of the intracellular signal transduction pathway induced in antigen presenting cells upon binding of the OX-2 T cell costimulatory molecule to its receptor on a T cell or other target cell. In addition to triggering a costimulatory signal in T cells, engagement of

the OX-2 costimulatory molecule with a receptor on T cells is likely to deliver distinct signals to the antigen presenting cell (the cell expressing the OX-2 T cell costimulatory molecule), e.g. through the cytoplasmic domain. By analogy to the B7 family of costimulatory molecules, it is believed that signals delivered through a novel OX-2 cytoplasmic domain may be of particular importance in the thymus, e.g., during positive selection of T cells during development, since structural forms of the analogous B7 costimulatory molecules comprising a novel cytoplasmic domain are known to be preferentially expressed in the thymus. For example, host cells expressing OX-2 T cell costimulatory molecules can be stimulated by crosslinking the OX-2 costimulatory molecule on the cell surface with an antibody and identifying intracellular signals and/or other cellular changes that are induced in the host cell in response to this crosslinking. In this manner, additional proteins or other cellular agents which play a role in the intracellular signal transduction pathway can be identified.

Additionally, an isolated OX-2 T cell costimulatory molecule of the invention can be used in methods for identifying other molecules (e.g., proteins) which interact with (bind to) the costimulatory molecule using standard in vitro assays (e.g., incubating the isolated costimulatory molecule with a cellular extract and determining by immunoprecipitation if any molecules present in the cellular extract bind to the costimulatory molecule). It is of particular interest to identify molecules which can interact with the cytoplasmic domain since such molecules may also be involved in intracellular signaling. For example, it is known that the cytoplasmic domains of many cell surface receptors can interact intracellularly with other members of the signal transduction machinery, e.g., tyrosine kinases.

The invention further provides a method for screening agents to identify a regulatory agent which up regulates or down regulates expression of an OX-2 T cell costimulatory molecule in vivo. The method involves contacting a cell which expresses or can be induced to express an OX-2 T cell costimulatory molecule with a putative regulatory agent and observing changes in the expression levels of a known or novel form of the OX-2 T cell costimulatory molecule in the cell. As used in reference to a regulatory agent, the term "up regulates" encompasses inducing the expression of a known or novel form of an OX-2 T cell costimulatory molecule by a cell which does not constitutively express such a molecule or increasing, by a statistically significant amount, the level of expression of a known or novel form of an OX-2 T cell costimulatory molecule by a cell which already expresses such a molecule. The term "down regulates" encompasses decreasing (to a statistically significant amount) or eliminating expression of a

known or novel form of an OX-2 T cell costimulatory molecule by a cell which already expresses such a molecule. The term "regulatory agent" is intended to include molecules which trigger an up regulatory or down regulatory response in a cell. For example, an agent can be a small organic molecule, a biological response modifier (e.g., a cytokine) or a molecule which can crosslink surface structures on the cell (e.g., an antibody). Expression levels of a known or novel OX-2 T cell costimulatory molecule(s) by the cell can be determined by, for example, detecting an mRNA transcript encoding the known or novel form of the OX-2 T cell costimulatory molecule in the cell. Once a novel form of OX-2 (e.g., an alternatively-spliced form) has been detected in a particular cell type, such cell types can be contacted with the putative regulatory agents and screened according to the above-noted screening methods of the invention to identify a regulatory agent which up regulates or down regulates expression of the novel form of OX-2.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patent publications, patents and patent applications cited throughout this application are incorporated in their entirety herein by reference.

EXAMPLES

EXAMPLE 1 - Identification of a Novel Costimulatory Agent

A. Experimental Methods

Animals. A Lewis rat was sacrificed and RNA was prepared from the thymus and spleen. Balb/c and DO.11 mice were sacrificed and spleens and thymuses harvested for the in vitro proliferation assays.

Antibodies and Peptide. Anti-ratOX-2 was purchased from Harlan Bioproducts for Science, Indianapolis, IN. Anti-B7-1 was purchased from Pharmingen, San Diego, CA. Murine CD28Ig and murine CTLA4Ig reagents were provided by Dr. T. Strom, Beth Israel Hospital, Boston, MA. Anti-CD3 (2C-11) was provided by Dr. J. Bluestone, University of Chicago, Chicago, IL. Chicken ovalbumin peptide, OVA-p (cOVA 323-339) is an IAd restricted peptide antigen (NH_2-ISQAVHAAHAEINEAGR-COOH; SEQ ID NO:18) which was synthesized at Stanford University.

Cytokines Measurements. In order to measure cytokine secretion from the cultured T cells, culture supernatants were collected at 24 hrs (to measure IL-2) and 48 hrs (to measure IFN-γ and IL-4) and assayed by ELISA (IL-2, IFN-γ and IL-4 mAbs, Pharmingen, San Diego, CA) according to the manufacturer's directions. Cytokine concentrations were determined using the

linear portion of the standard curve.

Transfections. CHO cell transfection was carried out by electroporation. In brief, 4 x 10^6 cells in 0.4 ml, were pulsed at 960 µF and 120 volts using a Biorad Gene Pulser (Biorad, Hercules, CA). The OX-2 expression plasmid was cotransfected with a plasmid encoding puromycin resistance at a ratio of 100:1, respectively in CHO cells or CHO cells previously transfected with the MHC Class II antigen, I-A^d (CHO-IAd, provided by Dr. Hans Reiser, Dana Farber Cancer Institute, Boston, MA). Transfected cells were selected in 12 µg/ml puromycin (Sigma, St. Louis, MO) for 4 days. Puromycin resistant CHO cells were cloned by limiting dilution, expanded and stained with anti-OX-2 antibody and analyzed by flow cytometry. High expressing clones and stable clones expressing OX-2 (CHO-OX-2) and I-A^d/OX-2 (CHO-IAd-OX-2) were identified by flow cytometry using the anti-OX-2 antibody. Stable B7-1 (CHO-B7-1) and I-A^d /B7-1 (CHO-IAd-B7-1) transfectants were provided by Dr. Gordon Freeman (Dana Farber Cancer Institute, Boston, MA).

Flow Cytometry. CHO cell transfectants were detached from tissue culture flasks with 2 mM EDTA in PBS and subsequently washed 3 times with DMEM. Cells were stained with anti-OX-2 (Harlan Bioproducts, Indianapolis), anti-B7-1 and anti-I-A^d antibodies (Pharmingen, San Diego), or with soluble receptor fusion proteins containing either murine CD28 or CTLA-4 linked to the murine IgG1 (CD28-Ig and CTLA-4-Ig, provided by Drs. Terry Strom and Peter Nickerson , Beth Israel Hospital, Boston, MA). Hamster IgG, mouse IgG1 and mouse IgG2a were used as isotype matched controls (Sigma, St. Louis, MO) for the anti-mB7-1 (hamster Ig), anti-I-A^d (mouse IgG2a), anti-OX-2 (mouse IgG1) and fusion proteins (mouse IgG2a). The secondary antibody reagents used were FITC goat anti-mouse and goat anti-hamster (Southern Biotechnology Associates, Inc., Birmingham, AL). Stained cells were analyzed on a FACSTAR flow cytometer (Becton Dickinson, Mountain View, CA).

Tissue Culture and Proliferation Studies. Lymphocytes were cultured in media (C10) comprised of RPMI supplemented with 10% heat-inactivated fetal calf serum (Sigma, St. Louis, MO), 5 x 10^{-5} M 2-mercaptoethanol, 2mM L-glutamine, 100 I.U./ml penicillin, 100 µg/ml streptomycin, 15 µg/ml gentamicin, 10mM HEPES (GIBCO, New York). T cells were prepared from single cell suspensions of spleen cells by nylon wool fractionation followed by purification by treatment with the M5-114 antibody (anti-class II, ATCC, Rockville, MD) and rabbit complement (Cedarlane Laboratories Limited, Ontario), as previously described (Freeman et al., *Science* 262:907-909, 1993), resulting in a preparation of approximately 85-95% purity. CD4+ T

cells were prepared as above by antibody-complement depletion of CD8$^+$ and MHCII$^+$ cells using the antibodies ADH4 and M5114, respectively. Thymocytes were prepared by mechanical dissociation of 4-5 week old thymus to make single cell suspensions, and then washing the cell suspensions three times in C10 lymphocyte plating media. CHO cells were grown to confluency and harvested with PBS containing 2mM EDTA, resuspended in PBS at 10^7 cells/ml and fixed in 0.4% paraformaldehyde for five minutes at room temperature. The fixation was stopped by adding an equal volume of 0.2M lysine in PBS followed by two times in C10 prior to use.

T cells (2×10^5) and paraformaldehyde treated CHO (5×10^4) cell transfectants were cocultured in C10 supplemented with either anti-CD3 (2C11) at 1 μg/ml for 3 days or with ovalbumin peptide (323-339) at 1 μg/ml. Blocking antibodies (1:10 dilution of supernatant), fusion protein (2 μg/ml) and isotype matched control (2 μg/ml) were preincubated with the CHO cell transfectants for 30 minutes prior to the addition of the T cells. Plates were pulsed with tritiated thymidine (New England Nuclear, Boston, MA) for the last 20 hours of a 3 day incubation. Incorporated radioactivity was measured by liquid scintillation counting.

Oligonucleotides. Primers O-228 (SEQ ID NO. 7) and O-229 (SEQ ID NO. 8) were used to amplify the ratOX-2 cDNA by RT-PCR from rat spleen and thymus DNA (described below). Cycling conditions using Taq DNA polymerase were 94°C for 4 minutes, followed by 35 cycles of 94°C for 45 seconds, 58°C for 45 seconds, 72°C for 2.5 minutes, followed by one extension at 72°C for 5 minutes. A single band was observed by agarose gel electrophoresis and cloned into the pT7-Blue plasmid vector (Novagen, Madison, WI). The sequence was confirmed from double stranded DNA plasmid templates by the dideoxy termination method using sequenase (USB,Cleveland, OH).

Primer O-228 (SEQ ID NO:7) contains a Kozak translational start sequence immediately upstream of the initiation methionine. Primer O-229 (SEQ ID NO:8) extends through the termination codon. Both primers have restriction sites placed at their ends for cloning purposes, e.g. for directional cloning into the pBK eukaryotic expression vector (Stratagene, La Jolla, CA).

O-228 (+) SENSE 5'-ATA GGATCC GCCGCCACC ATG GGCAGTCCGGTATTCAGGAGA-3'

BamHI Kozak initiation site Initiation codon

O-229 (-) ANTISENSE 5'-ATC TCTAGA TTA TTTCATTCTTTGCATCCCCTGT-3'

XbaI Termination codon

cDNA Library. A Balb/c spleen cDNA library in lambda gt11 (provided by Dr Lloyd Klickstein, Beth Israel Hospital, Boston, MA) was screened with the complete ratOX-2 cDNA. Hybridization was performed at 65°C for 24 hours and washed with increasing stringency up to 0.5% SSC/0.2%SDS. Blots were exposed to X-ray film for 48 hours. A single clone was obtained. This clone was subcloned into the plasmid pKSII (Stratagene, LaJolla, CA) for subsequent sequencing. This clone contained a deletion in the leader (signal) sequence probably due to a cloning artifact, but otherwise possessed excellent agreement to the published ratOX-2 sequence.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). Total cellular RNA was prepared from SWR/J mouse spleen and thymus using RNA-Stat-60 (Tel-Test "B", Inc. Friendswood, Texas). Random hexamer primed reverse transcription (RT) was performed with Superscript-RT (Gibco BRL, Gaithersburg MD) using 1-10 µg total RNA in a 20 µl reaction. All PCR reactions were performed in 25 µl volumes using a manual "hot start", wherein 10x deoxynucleotide triphosphates (dNTPs) were added to the samples at 80°C. Final reaction conditions were: 60 mM Tris-HCl, pH 8.5, 15 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 200 µM dNTPs, and 2 µg/ml each of the specific primers. Cycling conditions for all amplifications were 94°C, 4 minutes prior to 35 cycles of 94°C for 45 seconds, 58°C for 45 seconds, and 72°C for 3 minutes, followed by a final extension at 72°C for 7 minutes. The template for primary PCR was 2 µl of the RT reaction product and the template for secondary nested PCR was 1 µl of the primary PCR reaction product.

Rapid Amplification of cDNA Ends (RACE) Procedure. Polyadenylated RNA purified by two cycles of oligo-dT selection is obtained from CH1 B lymphoma cells, which express high levels of OX-2. Primers designed to the most 5' end of the cDNA are employed with the 5' RACE Kit (Gibco BRL, Gaithersburg, MD) according to the manufacturer's instructions. In brief, RNA is reverse transcribed with a gene-specific oligonucleotide, the cDNA purified, and a poly-dCTP tail is added with terminal deoxynucleotide transferase. PCR is performed using a nested primer and an oligonucleotide complementary to the poly-dCTP tail. PCR bands are cloned, sequenced, and correlated with the genomic sequences.

Oligonucleotide hybridization. Oligonucleotide(s) are 5' end-labeled with polynucleotide kinase and γ^{32}P-ATP. Hybridizations are carried out in 5X SSC and 5% SDS at 55°C overnight and subsequently are washed 3 times for 15 minutes with 2X SSC at 55°C. Blots are exposed to Kodak XAR-5 film with an intensifying screen at -80°C.

B. Results

(1) Cloning the rat OX-2 cDNA. The ratOX-2 cDNA was cloned with primers O-228 (SEQ ID NO: 7) and O-229 (SEQ ID NO: 8) from both Lewis rat thymus and spleen RNA. The sequence of the several cloned cDNA isolates contained a single conservative change from the published sequence of Clark et al., EMBO 4(1):113-118, 1985, at position 91 where a lysine was changed to an arginine. This sequence change could be due to a PCR artifact or alternatively to a strain difference between the Lewis rat used in this study and the Sprague-Dawley rat used by Clark et al. Despite this conservative single amino acid change, the cDNA was inserted into the pBK eukaryotic expression vector (Stratagene, LaJolla, CA) and cotransfected into either CHO or CHO-1Ad cells with a puromycin resistance gene-containing plasmid. CHO cells were placed under selection of 25 μg/ml puromycin and reached confluency in two weeks.

(2) FACS analysis. The resulting population was stained with anti-ratOX-2 antibody and analyzed by FACS. A positive population was sorted and cloned by limiting dilution. The CHO-ratOX-2 cells (and the CHO-1Ad-ratOX-2 cells), as expected, did not stain with either anti-B7-1 and anti-B7-2. To test whether ratOX-2 was sufficiently related to mB7-1 and mB7-2 to be defined as a B7-3, the ratOX-2 transfected cells were stained with the available soluble receptor-immunoglobulin fusion proteins of the B7:CD28/CTLA4 costimulatory pathway, namely, mCD28Ig and mCTLA4Ig. It is well established that human CTLA4Ig can stain mB7-1 and mB7-2 and thus it was anticipated that murine Ig fusion proteins would cross react with rat B7-like proteins. Under conditions which positively stained CHO-B7-1 and CHO-B7-2, the ratOX-2 transfectants failed to show any staining with either mCD28Ig and mCTLA4Ig. This result indicated that ratOX-2, despite some sequence similarity to mB7-1 and mB7-2, is not a member of the same costimulatory pathway and thus cannot be defined as a B7-3.

(3) Costimulation screening assay. Despite the FACS staining results we proceeded to investigate the ability of the ratOX-2 CHO transfectants to costimulate splenic $CD4^+$ T cells in a costimulation assay. CHO cells were inactivated by mild paraformaldehyde treatment (see methods). $CD4^+$ T cells were purified from older (>52 weeks) and younger (6-8 weeks) mice as previously described (H. Reiser and B. Benacerraf, PNAS 86:10069 (1989)). The primary signal was provided by soluble anti-CD3 at 1 μg/ml and the costimulatory signal provided by either untransfected CHO (as control) or CHO transfected with B7-1 or ratOX-2 (Figure 2A). The presence of the class II antigen $I\text{-}A^d$ on the CHO is irrelevant in experiments which employ soluble anti-CD3 as the primary signal. CHO cells (5×10^4) were added to the purified T cells (2

x 10^5) prior to the addition of anti-CD3. The assay was plated in a well triplicate format and proliferation was measured by tritiated thymidine uptake during the last 20 hours of a 3 day incubation. Costimulation by CHO-B7-1 was clearly observed, at approximately equal amounts, for both the responding T cells from young as well as old mice. Interestingly, CHO-ratOX-2 also costimulated both populations of responding T cells but was greater than 2 fold more pronounced when older T cells were used as the target cells. As expected, untransfected CHO cells did not costimulate the $CD4^+$ T cells.

The specificity of this costimulation was confirmed in a subsequent experiment using only young T cells and a variety of antibody blocking reagents (Figure 2B). Neither anti-B7-1, anti-B7-2 nor mCTLA4Ig was able to substantially abrogate ratOX-2 mediated costimulation whereas anti-ratOX-2 antibody blocked the proliferative response more than 80% under these conditions. These results demonstrate in a functional manner that ratOX-2 costimulation is independent of the B7:CD28/CTLA4 pathway.

Supernatants collected from the $CD4^+$ T cells stimulated with anti-CD3 and CHO cell transfectants were collected at 24 hours for IL-2 analysis and at 48 hours for IL-4 and IFN-γ analysis (Table I). Whereas IL-2, IL-4 and IFN-γ were readily detectable in the supernatants of T cells costimulated by CHO-IAd-B7-1, these cytokines were not detectable when CHO-IAd-OX-2 were used, despite the fact that the degree of T cell proliferation for both was comparable. This difference in cytokine production, in the presence of similar T cell proliferation serves to highlight a major difference in the functional outcomes between the OX-2 and the B7/CD28 costimulatory pathways.

Table I. Cytokine production by $CD4^+$ T cells following costimulation by OX-2 and B7-1 transfectants

	IL-2	IL-4	IFN-γ
CHO-IAd	<0.4	<4	<4
CHO-IAd-B7-1	42±3	133±15	16±5
CHO-IAd-OX-2	<0.4	<4	<4
T cells only	<0.4	<4	<4

IL-2 was measured at 24 hours while IL-4 and IFN-γ were measured at 48 hours. All values are indicated as units/ml. The limits of detection for IL-2, IL-4 and IFN-γ are 0.4, 4.0 and 4.0 units/ml, respectively.

In a parallel set of experiments performed in collaboration with Dr. Samuel Behar (Brigham and Women's Hospital, Boston, MA) it was determined that CHO-rat OX-2 cells costimulated human peripheral blood T cells in a manner similar to the stimulation of the mouse T cells as described above.

(a) Antigen specific costimulatory activity. We next appraised the ability of these CHO transfectants to costimulate T cell proliferation in an antigen dependent fashion (Figure 2C) - DO.11 TCR trangenic mice were used in this experiment for the vast majority of the T cells in this mouse have a single defined specificity against an chicken ovalbumin peptide (OVA-p, SEQ ID NO:18; see methods) in the context of I-A^d. The assay was plated in the same fashion except that OVA-p was used instead of anti-CD3 to provide the primary stimulation. The data clearly show that in the absence of a transfected costimulatory molecule, CHO cells did not induce proliferation by day 3. However, both B7-1 and ratOX-2 transfected CHO demonstrated antigen specific costimulatory capacity.

(b) Thymocyte costimulation. We appraised ratOX-2 and mB7-1 for their ability to costimulate thymocytes (Figure 3). Thymocytes were obtained from Balb/c mice (4-5 week old) and washed three times without further purification. Using the same cell ratios as in the previous studies, cells were given a primary stimulus of either anti-CD3 or OVA-p, followed by exposure to CHO, CHO-IAd-B7-1 or CHO-IAd-ratOX-2 transfectants.

OX-2 was also examined for its ability to provide costimulation to thymocytes from the DO11 TCR transgenic mice using CHO-IAd-OX-2 and CHO-IAd-B7-1 cells. Both CHO-IAd-OX-2 and CHO-IAd-B7-1 cells were capable of providing a costimulatory signal which led to an enhanced proliferation compared with the control CHO-IAd cells. This was true irrespective of whether anti-CD3 or ovalbumin peptide was used to provide the TCR signaling. Experiments using OX-2 and B7-1 transfectants in the absence of cotransfected I-A^d, to costimulate Balb/c thymocytes in the presence of anti-CD3, produced similar results.

(4) Sequence comparison. A comparison of the sequences of rat and mouse cDNAs indicated significant nucleotide identity/homology (compare SEQ ID NOs:3 and 5). The nucleotide sequence of the human OX-2 genomic DNA (as published in McCaughan et al., Immunogenetics 25:329-335, 1987) can also be compared to the rat and mouse sequences (compare the nucleic acid formed by SEQ ID NOs:1, 11, 12 and 19 with SEQ ID NOs:3 and/or 5). Based on manual alignment of the sequences (as described in Borriello et al., J Immunol., submitted), a comparison of the amino acid sequence of ratOX-2 to mB7-1 and mB7-2 indicated

significant homology, particularly in the Ig domain extracellular sequences (compare SEQ ID Nos:4, 9 and 10). These results are tabulated in Table II. A comparison of the Ig domains of OX-2 to murine B7-1 and B7-2 indicates homology that extends beyond what is expected of all Ig superfamily members and in fact has some residues in common with B7-1 and B7-2 which had previously been published as being specific to the B7 family of genes (Linsley et al. Protein Science 3:1341-1343 (1994)).

Table II: Amino acid sequence comparison between the MRC OX-2.. and murine B7-1 and B7-2 proteins.

	OX-2 x B7-1	OX-2 x B7-2	B7-1 x B7-2
IgV-like domain	25/32	24/39	27/40
IgC-like domain	23/35	20/33	32/44

Tabulated numbers indicate (percent identities)/(percent homologies)

A comparison of the amino acid sequences of the OX-2 antigens showed that the rat and murine proteins are more closely related to one another than to the human protein, consistent with the evolutionary relationship of these species (compare SEQ ID NOs:2, 4, and 6). Table III shows the numerical tabulation of the sequence comparison.

Table III: Numerical tabulation of the sequence comparison between rat, mouse and human OX-2 proteins

	leader	IgV	IgC	Tm	Cyt
rat x mouse	N.D.	92%	98%	100%	100%
rat x human	73%	76%	71%	96%	74%

The transmembrane (Tm) and cytoplasmic (Cyt) domains, in particular, show remarkable conservation and suggest a functional constraint during evolution. The conservation in the transmembrane domain suggests that OX-2 may have proceeded so as to preserve a critical function for that domain which may include the capacity to associate with other membrane proteins. The four conserved polar serine residues in the transmembrane also argue for

association of OX-2 with other proteins. Furthermore, the conserved cytoplasmic tail suggests either a conserved signaling function or an associative function with other proteins. This is in marked contrast to the cross species comparison of the B7-1 and B7-2 costimulatory proteins which, while maintaining a distinct homology in the extracellular domains, have little if any homology in the transmembrane or cytoplasmic domains.

The localization of the hOX-2 genes and those of B7-1 and B7-2 to chromosome 3 argue for a genetic locus rich in costimulatory molecules having a two Ig domain structure and suggests a genomic organization possibly derived from a primordial costimulatory molecule which may have duplicated several times, making it possible for the individual copies to acquire distinct immunologic roles. Thus the hOX-2 locus defines a "hot spot"' to search for additional costimulatory molecules in this class. A similar genomic hotspot is observed for the TNF-NGFR family of costimulators. By analogy, we believe that the receptor for OX-2 possesses a similar structure to CD28 and CTLA4 (a single IgV-like extracellular domain) and may also map to the genetic locus defined by CD28 and CTLA4.

C. Summary

We have demonstrated that ratOX-2 is a costimulatory protein for T cell activation. While ratOX-2 shares sequence homology to the well studied B7-1 and B7-2 costimulatory proteins, it failed to crossreact (stain) with either CTLA4Ig or CD28Ig. Furthermore, several features of the OX-2 costimulatory activity toward T cells and thymocytes are quite distinct from those observed in connection with B7 costimulation. These data define ratOX-2 as a novel costimulatory protein which functions through a B7:CD28/CTLA4 independent pathway.

The experiments presented herein were motivated in part by several similarities between ratOX-2 and the B7-1 and B7-2 costimulatory molecules. Aside from the structural similarities (discussed above), ratOX-2 protein has been reported to be present on some of the important antigen presenting cells in the immune system including B cells and endothelium, as well as on follicular dendritic cells. The presence of OX-2 on thymocytes also indicated to us that OX-2 may play a role in the development of thymocytes and possibly in the maturation of the TCR repertoire.

Both CHO-B7-1 and CHO-ratOX-2 costimulated $CD4^+$ T cells from spleen which had been stimulated with anti-CD3 at 1 µg/ml. While it is not possible to draw comparisons between the absolute costimulatory capacity of B7-1 versus that of ratOX-2 in a responding T cell population, it is instructive to note that when old T cells were used, the same CHO cells gave

quantitatively different results, namely, ratOX-2 exhibited twice the costimulatory capacity on old cells relative to young cells. In contrast, B7-1 costimulated both populations of T cells equally well. This suggests that the old CD4+ T cells had acquired a relatively greater responsiveness to ratOX-2 as compared to B7-1. Furthermore, both CHO-B7-1 and CHO-ratOX-2 costimulated DO.11 CD4+ T cells from spleen which had been stimulated with OVA-peptide at 10 μg/ml. This demonstrates that both B7-1 and ratOX-2 costimulated T cells in an antigen specific fashion. Whether the T cell populations responding to B7-1 and ratOX-2 costimulation are the same or distinct remains to be ascertained. In view of the foregoing results, it is clear that the ratOX-2 protein can be used to identify distinct functional parameters in T cell populations.

We examined another source of T cells (thymocytes) for their ability to respond to peptide. Thymocytes were stimulated either with anti-CD3 at 1 μg/ml or OVA-peptide at 10 μg/ml. CHO-B7-1 costimulated proliferation in either case. We previously observed that CD4+ spleen cells from the DO.11 mouse can be costimulated by CHO-ratOX-2 and anti-CD3. This suggests that a developmental difference between the TCR transduction pathway in immature thymocytes versus that found in mature splenocytes. As the antigen-MHC complex is the more physiologic form of TCR engagement relative to anti-CD3, it is reasonable to presume that ratOX-2 can act in the thymus, as well as in periphery. These results are consistent with the hypothesis that costimulation may be involved in T cell maturation in the thymus.

An analysis of the cytokine profiles from the OX-2 and B7-1 costimulated CD4+ T cells highlights certain functional differences between the engagement of these two costimulatory molecules. IL-2, IL-4 and IFN-γ levels were readily measured after B7-1 mediated costimulation, while these cytokines were below measurable levels following OX-2 mediated costimulation, even though the levels of proliferation were comparable. While these cytokine profiles clearly indicate a functional difference between these two pathways, a possible role for IL-2 has still to be excluded, by blocking the IL-2 receptor during the course of these costimulation assays. The notable absence of detectable IL-2 after OX-2 costimulation could have implications for T cell anergy. It will be possible now to determine the functional implications of OX-2 costimulation, such as the ability to prevent anergy. Other cytokines with effects on T cell, such as IL-1, IL-6, IL-9 and IL-15 can be examined to determine the functional basis for the OX-2 mediated proliferation of T cells. The distinct cytokine profiles between OX-2 and B7-1 mediated costimulation however, support the hypothesis that OX-2 is part of a T cell

costimulatory pathway functionally distinct from the B7/CD28 pathway. OX-2 expression on the endothelium suggests that OX-2 may be involved in the events by which T cells are activated at sites of inflammation, by their interaction with the endothelium, that lead to transmigration and downstream effector functions. Strong expression on the follicular dendritic cells also suggests a possible role for OX-2 in the interaction of B-lymphocytes and professional antigen presenting cells.

EXAMPLE 2 - Identification of a Novel OX-2 Signal Peptide Domain

cDNA fragments corresponding to the 5' and 3' ends of naturally-occurring human, rat or mouse OX-2 mRNA transcripts were prepared by using a commercially available kit (Marathon RACE Kit, CLONTECH Laboratories, Palo Alto, CA) according to the manufacturer's instructions. Using this procedure, polymorphisms at both the 5' and 3' ends of the OX-2 cDNAs were identified. A polymorphism at the 3' end involves the 3' untranslated region and may be relevant to post-transcriptional control of OX-2 expression. A polymorphism at the 5' end involves the use of an alternative signal sequence which is approximately 75 base pairs longer than the known signal exon. The additional amino acids at the amino terminus of the protein may have functional ramifications for the interaction of this OX-2 variant with its receptor.

EXAMPLE 3 - Identification of alternatively-spliced forms of OX-2 having a structural domain deleted.

Reverse-transcriptase polymerase chain reaction is used to amplify human, rat or mouse cDNA fragments derived from human tumor cells, peripheral blood and spleen cell RNA. Oligonucleotide primers useful for PCR are as follows. An exemplary primer pair which bridges the IgV to the transmembrane/cytoplasmic domain and hence is useful for detecting an alternatively spliced OX-2 which lacks IgC domain is:

Primer 1, sense, IgV exon, spans amino acids 3-9 (Clark et al., ibid., numbering scheme)

5'- GAA GTG GTG ACC CAG GAT GAA -3' (SEQ ID NO: 14)

Primer 2, antisense, transmembrane exon, spans amino acids 222-228 (Clark et al., ibid., numbering scheme)

5'- GTA TAA TAA GAT GGA GAT CAA -3' (SEQ ID NO: 15)

Using the foregoing general procedure , a cDNA fragment is detected which comprises a nucleotide sequence encoding a human OX-2 molecule in which the signal peptide domain is spliced directly to the IgC-like domain (i.e., the IgV-like domain is deleted). Alternatively, using

the above-identified PCR oligonucleotide primers, another cDNA fragment is detected with comprises a nucleotide sequence encoding a human OX-2 molecule in which the IgV-like domain is spliced directly to the transmembrane/cytoplasmic domain (i.e., the IgC-like domain is deleted). This protein is referred to herein as an IgV-like isoform of OX-2 or as a "pOX-2" ("p" is for "partial" OX-2 molecule). To examine the functional activity of the IgV-like isoform of OX-2, its cDNA is cloned into an expression vector, pBK-CMV, in which transcription of the cDNA is placed under the control of the CMV promoter. The expression vector is cotransfected into Chinese Hamster Ovary (CHO) cells, along with a puromycin resistance gene, and drug resistant clones are selected. The resultant clones expressing the IgV-like isoform of human OX-2 on their surface are referred to herein as CHO.pOX-2 cells.

Expression of the IgV-like isoform of hOX-2 on the surface of the CHO-pOX-2 cells is confirmed by FACS analysis using an OX-2 receptor or anti-OX-2 antibody as the primary staining reagent. This reagent stains the CHO-pOX-2 cells. Positive staining of CHO-pOX-2 with the OX-2 receptor indicates that the IgV-like isoform of hOX-2 is capable of interacting with the OX-2 receptor.

The ability of the IgV-like isoform of hOX-2 on CHO-pOX-2 cells to deliver a costimulatory signal to T cells is tested in standard T cell proliferation and interleukin-2 (IL-2) production assays, as well as in the other above-described costimulation screening assays. T cells that receive a primary activation signal are stimulated to produce IL-2 when incubated with either CHO-OX-2 cells or CHO-pOX-2 cells but not when incubated with untransfected CHO cells. Similar results are observed when T cell proliferation is assayed as an indicator of T cell costimulation.

EXAMPLE 4 - Preparation and characterization of OX-2 antibodies and fusion proteins

A. Antibodies to OX-2. To facilitate analysis of OX-2 expression in the mouse and rat systems, novel antibodies were developed.

Monoclonal antibodies to murine OX-2 were prepared by standard methodologies using a mOX-2Ig fusion protein as an antigen. A panel of ten monoclonal antibodies which bind specifically to mOX-2 were characterized for utility in FACS analysis of cells expressing mOX-2. Two of the ten monoclonal antibodies stained murine splenocytes sufficiently to permit detection by FACS. The other 8 monoclonal antibodies were not as useful in FACS analysis but can be used for immunoprecipitation or Western blot analysis. A monoclonal antibody also was developed against rat OX-2 using standard methodologies and rOX-2-Ig fusion protein as an

immunogen. This antibody is structurally and functionally distinct from the commercially available rat OX-2 antibody.

B. OX-2 fusion proteins. Soluble variants (OX-2-Ig fusion proteins) of murine, rat and human OX-2 molecules were prepared (mOX-2-Ig, rOX-2-Ig and hOX-2-Ig, respectively). These molecules are analogous to CTLA4-Ig soluble costimulatory molecule prepared by Brian Seed (Zettlmeissl et al. (1990) DNA and Cell Biol. 9(5):347-353) and used to block costimulation of T cells.

The following primers were used to generate PCR products from which BamHI fragments having (GGATCC) ends could be formed and spliced into an Ig expression vector available from Dr. Brian Seed (Massachusetts General Hospital, Boston, MA).

Mouse/rat OX-2

For expression (SEQ ID NO:):

O-228 (+) 5'-ATAGGATCCGCCGCCACCATGGGCAGTCCGGTATTCAGGAGA-3'

For Ig fusion (SEQ ID NO:20):

309 (-) 5'-AGGATCCTTGTCCAGACTCTGCTT-3'

Human OX-2

For expression (SEQ ID NO:21):

226 (+) 5'-ATAGGATCCGCCGCCACCATGCCCTTCTCTCATCTGTCT-3'

For Ig fusion (SEQ ID NO:22):

FB23 (-) 5'-ATGGATCCCCTTTGTTGACGGTTTGCTT-3'

The protein sequences produced in this manner have only slight amino acid differences from the composite native proteins at the junction formed by BamHI ligations.

For the mOX-2-Ig and rOX-2-Ig, the amino acid sequence of the mOX-2 or rOX-2 portion is as listed (SEQ ID NOs:4 and 6) except that after the DK motif at the end of the IgC domain, there is the amino acid sequence DPR, unrelated to mOX-2, rOX-2 or Ig, followed by the human IgG1 heavy chain hinge region and the CH2 and CH3 domains.

For the hOX-2-Ig, the amino acid sequence of the hOX-2 portion is as listed (SEQ ID NO:2) except that after the NK motif at the end of the IgC domain, there is the amino acid sequence GDPR (SEQ ID NO:23), unrelated to hOX-2 or Ig, followed by the human IgG1 heavy

chain hinge region and the CH2 and CH3 domains.

The mOX-2Ig prepared as described above was found to inhibit the function of lymphocytes in several *in vitro* assays, including an allogeneic mixed lymphocyte reaction (allo-MLR) and anti CD3 induced proliferation of mouse splenocytes. In an allo-MLR, unadulterated lymphocytes of one genetic background are cultured with inactivated cells of a different genetic background. After several days of incubation the unadulterated cells will respond against the foreign cells by proliferating, producing cytokines and developing specific cytotoxic effector functions. When mOX-2-Ig was added to an allo-MLR at 25μg/ml, proliferation of the unadulterated cells was reduced greater than 5 fold compared to an allo-MLR to which a control Ig was added. Addition of mOX-2-Ig to an anti-CD3 splenocyte proliferation assay reduces proliferation of splenocytes to approximately the same extent as does mCTLA4Ig.

Similarly, mOX-2Ig inhibited an allo-MLR induced proliferation in a human system, i.e., the murine OX-2 fusion protein cross-reacts with the human receptor for OX-2. Thus, the mOX-2 Ig also can be used to detect OX-2 receptors by FACS analysis as described in Example 1 and can be used in adhesion assays to determine whether the OX-2 receptor is expressed on certain cell types. For example, the murine OX-2 fusion protein has been used to demonstrate that ConA/anti-CD3 activated but not resting splenocytes adhere to mOX-2-Ig coated plastic wells. Thus at least one receptor for OX-2 appears to be an activation induced surface molecule.

EXAMPLE 5 - OX-2 specifically stimulates Th1 T cells.

An adhesion assay was performed to assess the specificity of T cell stimulation by OX-2. Such assays are performed in general as follows. The fusion protein (mOX-2-Ig) is purified from supernatants of the stable CHO cell transfectants using a Protein A sepharose column. The supernatants are passed over the column, the column is washed with phosphate buffered saline (PBS) and the fusion protein is eluted in a 0.1M glycine buffer at pH 3.5. The fusion protein elutes in the first 3ml and the eluate is rapidly neutralized with Tris base.

This material is standardized for IgG1 content using standard curves of human IgG1 protein. This material is then plated onto a polystyrene surface which binds proteins. The incubation proceeds for 1 hour at 37°C and the surface is then washed and blocked with 1% bovine serum albumin (BSA) in PBS. The cells to be tested are then placed on the plastic and allowed to settle to the surface for 1 hour at 37°C. The surface is gently washed to remove cells which are not bound while leaving the bound cells undisturbed. This step is empirically established and dependent on the strength of the interaction being tested.

Bound cells are then counted and reported either by cell number per unit area, or percent input cells bound. A control fusion protein is always used to control for nonspecific binding of which there is none in the assays performed to date.

An adhesion assay indicated that a Th1 T cell clone, but not a Th2 T cell clone, constitutively expressed the receptor for OX-2. Unstimulated spleen cells did not bind to the murine OX-2 fusion protein. Thus, the receptor for OX-2 may represent a costimulatory receptor which is differentially used by the Th1 and Th2 helper T cell subsets.

In support of the Th1- specific nature of OX-2 costimulation, rOX-2 transgenic mouse strain was developed. The overexpression of a costimulatory protein is essentially a pharmacalogic experiment to address the question of what effect an overdose of the costimulatory signal may have *in vivo*.

The experiment parallels a similar approach used for B7-1 (Sethna et al., Immunity 1(5):415-421, 1994). A rOX-2 cDNA was cloned by PCR amplification as described above, BamHI digestion of the PCR product and ligation into an expression vector. The vector contains the immmunoglobulin promoter-enhancer which drives expression of the cDNA in B cells and/or T cells. The vector also contains growth hormone 3' untranslated sequences (including poly A sequence) to stabilize the mRNA: these sequences are thought to promote higher expression levels of the cDNA cloned into the vector.

The mouse was generated using standard transgenic techniques involving the injection of the rOX-2 expression vector DNA into fertilized eggs. Transgenic mice were selected by PCR using oligos O-228 and O-229. PCR with these oligonucleotides produced a 0.9kb rOX-2 cDNA amplification product which is not present in wildtype mice. The transgenic mice and their wildtype littermates were then examined for histology. One physical finding is that the thymuses of the transgenic mice were half the size of the the thymuses of the wildtype mice

A rOX-2 transgenic mouse was immunized with a haptenated model protein antigen called TNP-KLH. TNP (trinitrophenol) is a small organic molecule and KLH (keyhole limpet hemocyanin) is a large protein. This commonly used antigen was used to immunize animals intravenously. Serum from these animals was assayed at day 7 post-immunization and the levels of TNP specific antibody titers were assayed using a standard ELISA technique. After 7 days, the types of IgG specific for the model protein antigen were characterized. The ratio of IgG2a:IgG1 was increased 3-8 fold in the 7 days into a primary response of the transgenic mice. This result is consistent with an increase in the ratio of Th1:Th2 activity in the immune response.

as Th1 activity promotes IgG2a immunoglobulin isotype switching while Th2 promotes IgG1 switching. This result is consistent with the result that OX-2 interacts preferentially with a receptor on Th1 cells.

EXAMPLE 6 - Isolation of mouse OX-2 genomic clones.

To isolate genomic DNA clones corresponding to the OX-2 cDNA, an OX-2 probe was hybridized to a mouse genomic library according to standard procedures. Clones were isolated and sequenced according to standard procedures to confirm the identity of the genomic clones with OX-2 cDNA.

EXAMPLE 7 - Preparation of antibodies to the OX-2 receptor and characterization of the OX-2 receptor.

Monoclonal antibodies to the OX-2 receptor are developed using the following exemplary procedure. Hamsters are immunized with a Th1 clone (e.g., AE7) that constitutively expresses the receptor. Spleen cells from the immunized hamster are fused with myeloma cells to create hybridoma fusion cells using standard techniques. The hybridoma cells are cultured and supernatants of the hybridoma cultures are screened for the ability to specifically inhibit the adhesion reaction of Th1 cells to the mOX-2-Ig coated plastic wells. All inhibiting antibodies must be directed against either mOX-2-Ig on the substrate or OX-2 receptor(s) on the surface of the Th1 clone. As mOX-2-Ig is plated in excess, it is improbable that all of the mOX-2-Ig can be blocked by hybridoma supernatant. In contrast, the use of a limited number of Th1 cells and preincubation of the Th1 cells with the hybridoma supernatant permits the limited number of receptor(s) to be specifically blocked, thus inhibiting the adhesion to the mOX-2-Ig coated wells.

The development of anti-OX-2 receptor monoclonal antibodies permits characterization of the receptor(s), both at the biochemical level by immunoprecipitation and western blotting, and at the functional level using *in vitro* and *in vivo* blocking experiments. The development of anti-OX-2 receptor monoclonal antibodies also permits cloning of the OX-2 receptor itself. The cloning of the OX-2 receptor is achieved by expression cloning from a cDNA library made from the mRNA of activated spleen cells or the Th1 cells used previously in generating the anti-OX-2 receptor monoclonal antibodies. The OX-2 receptor is also cloned by purification of the antigen recognized by the anti-OX-2 receptor monoclonal antibodies, followed by microsequencing of the antigen and PCR amplification of the cDNA using degenerate oligonucleotides as PCR primers. Alternatively, the mOX-2-Ig fusion protein may interact sufficiently tightly with the receptor to directly permit an expression cloning strategy.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

A Sequence Listing is presented below and is followed by what is claimed.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Borriello, Francescopaolo

Sharpe, Arlene H.

(ii) TITLE OF INVENTION: OX-2 COSTIMULATORY MOLECULE

(iii) NUMBER OF SEQUENCES: 23

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Wolf, Greenfield & Sacks, P.C.

(B) STREET: 600 Atlantic Avenue

(C) CITY: Boston

(D) STATE: MA

(E) COUNTRY: USA

(F) ZIP: 02210

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 60/008,754

(B) FILING DATE: 08-DEC-1995

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Plumer, Elizabeth R.

(B) REGISTRATION NUMBER: 36,637

(C) REFERENCE/DOCKET NUMBER: B0801/7058

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 617-720-3500

(B) TELEFAX: 617-720-2441

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 210 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: exon

(B) LOCATION: 59..140

(ix) FEATURE:

(A) NAME/KEY: sig_peptide

(B) LOCATION: 59..136

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 210

(D) OTHER INFORMATION: /note= "gap of approximately 3.4 kb between SEQ ID NO:1 and SEQ ID NO:11"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GATCAGGATG CCCTTCTCTC ATCTGTCTAC CTACAGCCTG GTTTGGGTCA TGGCAGCAGT 120

GGTGCTGTGC ACAGCACAAG GTAAAGAAAC TCAATTCCCC TGCTTGGAGC CCAGCAAACA 180

CAATTTCTGG GGTGAAGACA TTTAGCCAGA 210

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 278 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION: 1..33

(D) OTHER INFORMATION: /label= signal_peptide

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 34..278

(D) OTHER INFORMATION: /note= "mature protein"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Ser Leu Val Trp Val Met Ala Ala Val Val Leu Cys Thr Ala Gln Val
-15 -10 -5

Gln Val Val Thr Gln Asp Glu Arg Glu Gln Leu Tyr Thr Thr Ala Ser
1 5 10 15

Leu Lys Cys Ser Leu Gln Asn Ala Gln Glu Ala Leu Ile Val Thr Trp
20 25 30

Gln Lys Lys Lys Ala Val Ser Pro Glu Asn Met Val Thr Phe Ser Glu
35 40 45

Asn His Gly Val Val Ile Gln Pro Ala Tyr Lys Asp Lys Ile Asn Ile
50 55 60

Thr Gln Leu Gly Leu Gln Asn Ser Thr Ile Thr Phe Trp Asn Ile Thr
65 70 75

Leu Glu Asp Glu Gly Cys Tyr Met Cys Leu Phe Asn Thr Phe Gly Phe
80 85 90 95

Gly Lys Ile Ser Gly Thr Ala Cys Leu Thr Val Tyr Val Gln Pro Ile
100 105 110

Val Ser Leu His Tyr Lys Phe Ser Glu Asp His Leu Asn Ile Thr Cys
115 120 125

Ser Ala Thr Ala Arg Pro Ala Pro Met Val Phe Trp Lys Val Pro Arg
130 135 140

Ser Gly Ile Glu Asn Ser Thr Val Thr Leu Ser His Pro Asn Gly Thr
145 150 155

Thr Ser Val Thr Ser Ile Leu His Ile Lys Asp Pro Lys Asn Gln Val
160 165 170 175

Gly Lys Glu Val Ile Cys Gln Val Leu His Leu Gly Thr Val Thr Asp
180 185 190

Phe Lys Gln Thr Val Asn Lys Gly Tyr Trp Phe Ser Val Pro Leu Leu
195 200 205

Leu Ser Ile Val Ser Leu Val Ile Leu Leu Val Leu Ile Ser Ile Leu
210 215 220

Leu Tyr Trp Lys Arg His Arg Asn Gln Asp Arg Gly Glu Leu Ser Gln
225 230 235

Gly Val Gln Lys Met Thr
240 245

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2216 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

(A) NAME/KEY: sig_peptide

(B) LOCATION: 25..123

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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GAAGTGGTGA CCCAGGATGA AAGAAAGCTG CTGCACACAA CTGCATCCTT ACGCTGTTCT 180

CTAAAAACAA CCCAGGAACC CTTGATTGTG ACATGGCAGA AAAAGAAAGC CGTAGGCCCA 240

GAAAACATGG TCACTTACAG CAAAGCCCAT GGGGTTGTCA TTCAGCCCAC CTACAAAGAC 300

AGGATAAACA TCACTGAGCT GGGACTCTTG AACACAAGCA TCACCTTCTG GAACACAACC 360

CTGGATGATG AGGGTTGCTA CATGTGTCTC TTCAACATGT TTGGATCTGG GAAGGTCTCT 420

GGGACAGCTT GCCTTACTCT CTATGTACAG CCCATAGTAC ACCTTCACTA CAACTATTTT 480

GAAGACCACC TAAACATCAC GTGCTCTGCA ACTGCCCGCC CAGCCCCTGC CATCTCCTGG 540

AAGGGCACTG GGTCAGGAAT TGAGAATAGT ACTGAGAGTC ACTCCCATTC AAATGGGACT 600

ACATCTGTCA CCAGCATCCT CCGGGTCAAA GACCCCAAAA CTCAGGTTGG AAAGGAAGTG 660

ATCTGCCAGG TTTTATACTT GGGGAATGTG ATTGACTACA AGCAGAGTCT GGACAAAGGA 720

TTTTGGTTTT CAGTCCCACT GCTGCTGAGC ATTGTTTCTC TGGTAATTCT TCTGGTCTTG 780

ATCTCCATCT TATTATACTG GAAACGGCAC CGAAATCAGG AGCGGGGTGA GTCATCACAG 840

GGGATGCAAA GAATGAAATA AGAGCTCTAG AGAAATTATA TCAGAACCCT GAACGTGTTT 900

CCCTGGTCTA CTTGAATCTG ATGTTAAAGA AAAGCAGGAG GGAAAAGGTC ATTCTCCATA 960

GGACCTAAAA AAGAGCAAAA GATGCAGAAG GAAGCCTGTG AGGGATTTGA CTTTTTGCTG 1020

CTGTCCCGGG TCCTCAGTAT TTACATTCCA AGAGGAAGTT GTGTGCCTCG GGTCTGTTGC 1080

AGGACTTGAT TTTTGTGGAG CAATGCAGCG TCATTGCTGT TAGAAAGGCA CCAGACTTAG 1140

AATCACCAGT GCCAAGCCGG CTCGCAGACC GACTAGGGCT CCCACCGGAG GGACAAATCA 1200

TAGTCAACTT ACCTCACAGA GCTTCCTGGT CTTTATACAA AGTAGAAAGG AGTGGGACCA 1260

GAAAATTTGC CATGTCTGAG ATCTGATGGA ATTTTAGGAA GAAAACTGAA GAGCGATCAA 1320

AAGAAGAAGA GAGAACACAG AAGGGTCAAA GAGCTTCGGA GAGTACCTTT TGCCTTTCTG 1380

TTGGGGTCCC ACCTCTGGTT TTGTTCTTAG GTCCACCAGT TTGTTTCCCT GTTGTTTGAG 1440

TATCTAATTG ACTACCTGCT ACTGTTCCGC TGATTGTTGG CCTTGCTAAA ATCCCTGATT 1500

CCCCTGCCGT TCTCTATGTG CTTCTATGAG GGTTACTATG ATGAAAATAG AGAAGAATTT 1560

AGTGTGAAGT AACATTGGCA ACCGTAATGT GTCCATTTAA CTTATTTTTA TAGCACGTAG 1620

GCAAATACCG TTAGTCTTAG CAAGTAGTTC ACATCTTTAC AAAAGCATGC TTTCCCTGTC 1680

CATTTGGCCC AGGATATCAC CCACTTTGAG GCCATTCTGA ATCCTGTCTC GTGTAACGAT 1740

AATATATTAT GAAAACAGAT GTGTTAAGAA TTTCCTGTAC AGCAGTCAGT TGTATATTCT 1800

CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CTCTCTCAAC TTCTTTTTCT 1860

GTGACTTTAT TTTTCACAAA GAGAAGGCAA CTCTGGAATA CAATCGCTTT GTTCTGAAGA 1920

CATTTGTGGA CATCTTAACC CTTTGACAGA ACTAGTGACG TTGTTTTCTG TATCTTTTGC 1980

TTCATCTGTC TCTGTAGAGT GACCTAGGAA TTCAAGTGTA AGTTGTTTTC ATTGTCAAAC 2040

TCGATATTTA TATACTTGGT ATGCTTTTCA TGTGTTATTT AATTCCGTAT AATTTTCCTA 2100

TATTTGTATT AAAATACTGA GCAATTAAAA GTGTCAACTA AATAGTTGAT GTGTGACATT 2160

CCCTTGAGAA ATATAGAAAT AAAGAATAAC ACTTGGATTT TTTTTTCTCT GTATTC 2216

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 278 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION: 1..33

(D) OTHER INFORMATION: /label= signal peptide

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 34..278

(D) OTHER INFORMATION: /note= "mature protein"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Gly Ser Pro Val Phe Arg Arg Pro Phe Cys His Leu Ser Thr Tyr
                -30                 -25                 -20
Ser Leu Leu Trp Ala Ile Ala Ala Val Ala Leu Ser Thr Ala Gln Val
            -15                 -10                 -5
Glu Val Val Thr Gln Asp Glu Arg Lys Leu Leu His Thr Thr Ala Ser
    1               5                   10                  15
Leu Arg Cys Ser Leu Lys Thr Thr Gln Glu Pro Leu Ile Val Thr Trp
                20                  25                  30
Gln Lys Lys Lys Ala Val Gly Pro Glu Asn Met Val Thr Tyr Ser Lys
            35                  40                  45
Ala His Gly Val Val Ile Gln Pro Thr Tyr Lys Asp Arg Ile Asn Ile
        50                  55                  60
Thr Glu Leu Gly Leu Leu Asn Thr Ser Ile Thr Phe Trp Asn Thr Thr
    65                  70                  75
```

Leu Asp Asp Glu Gly Cys Tyr Met Cys Leu Phe Asn Met Phe Gly Ser
80 85 90 95

Gly Lys Val Ser Gly Thr Ala Cys Leu Thr Leu Tyr Val Gln Pro Ile
100 105 110

Val His Leu His Tyr Asn Tyr Phe Glu Asp His Leu Asn Ile Thr Cys
115 120 125

Ser Ala Thr Ala Arg Pro Ala Pro Ala Ile Ser Trp Lys Gly Thr Gly
130 135 140

Ser Gly Ile Glu Asn Ser Thr Glu Ser His Ser His Ser Asn Gly Thr
145 150 155

Thr Ser Val Thr Ser Ile Leu Arg Val Lys Asp Pro Lys Thr Gln Val
160 165 170 175

Gly Lys Glu Val Ile Cys Gln Val Leu Tyr Leu Gly Asn Val Ile Asp
180 185 190

Tyr Lys Gln Ser Leu Asp Lys Gly Phe Trp Phe Ser Val Pro Leu Leu
195 200 205

Leu Ser Ile Val Ser Leu Val Ile Leu Leu Val Leu Ile Ser Ile Leu
210 215 220

Leu Tyr Trp Lys Arg His Arg Asn Gln Glu Arg Gly Glu Ser Ser Gln
225 230 235

Gly Met Gln Arg Met Lys
240 245

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1264 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

(A) NAME/KEY: sig_peptide

(B) LOCATION: 38..46

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 38..794

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCGAGAAGCT GGTGTCTAGC TGCGNCCCAG AGCAAGGATG GGCAGTCTGT GGAAGTTGGT 60

GACCCAGGAT GAAAGAAAGG CNCTGCACAC AACTGCATCC TTACGATGTT CTCTAAAGAC 120

ATCCCAGGAA CCCTTGATTG TGACATGGCA GAAAAAGAAA GCCGTGAGCC CAGAAAACAT 180

GGTCACCTAC AGCAAAACCC ATGGGGTTGT AATCCAGCCT GCCTACAAAG ACAGGATAAA 240

TGTCACAGAG CTGGGACTCT GGAACTCAAG CATCACCTTC TGGAACACAA CATTGGAAGA 300

TGAGGGTTGC TACATGTGTC TCTTCAACAC GTTTGGTTCT CAGAAGGTCT CAGGAACAGC 360

TTGGCCTTAC CCTCTATGTA CAGCCCATAG TACACCTTCA CTACAACTAT TTTGAAGACC 420

ACCTAAACAT CACTTGCTCT GCGACTGCCC GCCCAGCCCC TGCCATCTCC TGGAAGGGCA 480

CTGGGACAGG AATTGAGAAT AGTACCGAGA GTCACTTCCA TTCAAATGGG ACTACATCTG 540

TCACCAGCAT CCTCCGGGTC AAAGACCCCA AAACTCAGGT TGGAAAGGAA GTGATCTGCC 600

AGGTTTTATA CCTGGGGAAT GTGATTGACT ACAAGCAGAG TCTGGACAAA GGATTTTGGT 660

TTTCAGTTCC ACTGTTGCTA AGCATTGTTT CTCTGGTAAT TCTTCTGGTC TTGATCTCCA 720

TCTTACTATA CTGGAAACGT CACCGAAATC AGGAGCGGGG TGAATCATCA CAGGGGATGC 780

AAAGAATGAA ATAAGAGCTC TAGAGAAATT ATACAGACCC TGAACGTGTT TCCCTGGTCT 840

ACTTGAATCT GATGTGAAAG AAAAGCAGGA GGGAAAAGGC CATTCTCCAT AGGACCTAAG 900

GAGAGCAAAA GACCAGACNC GAGCCTGTGC GGGATTTGAC TTTTTGCTGT TGTCCCAGGT 960

CCTCGGTGTT TGCATTCCAA GAGGAAGTCG AGTGCCTCGG GTCTGTTGTA GGACTTGATT 1020

TTTTTTTTTT TTTGTAGAGC AATGCAGTGC CATGCTGTTA GAAAGNCTCC AGACTTAGAA 1080

CCACCAGTGC CAANCCAGCT CTCAGACCGA CTACCCGGAG GAACAAATCG TAGTCAACTT 1140

ACCTCACAGA GCTCTCTGGT CCTTACACAA AGTAGAAAGG AGTGGNNNNN GAAAATTGGC 1200

CATGTCTGAA ATCTGATGGA ATTTTTAGGA AGAAAACTGA AGAATAAGNA AAAAAAAAAA 1260

AAAG 1264

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 278 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION: 1..31

(D) OTHER INFORMATION: /label= signal_peptide

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 32..278

(D) OTHER INFORMATION: /note= "mature protein"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Gly Ser Xaa Val Phe Arg Arg Pro Phe Cys His Leu Ser Thr Tyr
-30 -25 -20

Ser Leu Leu Trp Ala Ile Ala Ala Val Ala Leu Ser Thr Ala Xaa Xaa
-15 -10 -5 1

Xaa Xaa Val Thr Gln Asp Glu Arg Lys Ala Leu His Thr Thr Ala Ser
5 10 15

Leu Arg Cys Ser Leu Lys Thr Thr Gln Glu Pro Leu Ile Val Thr Trp
20 25 30

Gln Lys Lys Lys Ala Val Ser Pro Glu Asn Met Val Thr Tyr Ser Lys
35 40 45

Thr His Gly Val Val Ile Gln Pro Ala Tyr Lys Asp Arg Ile Asn Ile
50 55 60 65

Thr Glu Leu Gly Leu Trp Asn Thr Ser Ile Thr Phe Trp Asn Thr Thr
70 75 80

Leu Glu Asp Glu Gly Cys Tyr Met Cys Leu Phe Asn Thr Phe Gly Ser
85 90 95

Gln Lys Val Ser Gly Thr Ala Cys Leu Thr Leu Tyr Val Gln Pro Ile
100 105 110

Val His Leu His Tyr Asn Tyr Phe Glu Asp His Leu Asn Ile Thr Cys
115 120 125

Ser Ala Thr Ala Arg Pro Ala Pro Ala Ile Ser Trp Lys Gly Thr Gly
130 135 140 145

Thr Gly Ile Glu Asn Ser Thr Glu Ser His Ser His Phe Asn Gly Thr
150 155 160

Thr Ser Val Thr Ser Ile Leu Arg Val Lys Asp Pro Lys Thr Gln Val
165 170 175

Gly Lys Glu Val Ile Cys Gln Val Leu Tyr Leu Gly Asn Val Ile Asp
180 185 190

Tyr Lys Gln Ser Leu Asp Lys Gly Phe Trp Phe Ser Val Pro Leu Leu
195 200 205

Leu Ser Ile Val Ser Leu Val Ile Leu Leu Val Leu Ile Ser Ile Leu
210 215 220 225

Leu Tyr Trp Lys Arg His Arg Asn Gln Glu Arg Gly Glu Ser Ser Gln
230 235 240

Gly Met Gln Arg Met Lys
245

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 42 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATAGGATCCG CCGCCACCAT GGGCAGTCCG GTATTCAGGA GA 42

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATCTCTAGAT TATTTCATTC TTTGCATCCC CTGT 34

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 306 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION: 1..36

(D) OTHER INFORMATION: /label= signal_peptide

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 37..306

(D) OTHER INFORMATION: /note= "mature protein"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Ala Cys Asn Cys Gln Leu Met Gln Asp Thr Pro Leu Leu Lys Phe
-35 -30 -25

Pro Cys Pro Arg Leu Ile Leu Leu Phe Val Leu Leu Ile Arg Leu Ser
-20 -15 -10 -5

Gln Val Ser Ser Asp Val Asp Glu Gln Leu Ser Lys Ser Val Lys Asp
1 5 10

Lys Val Leu Leu Pro Cys Arg Tyr Asn Ser Pro His Glu Asp Glu Ser
15 20 25

Glu Asp Arg Ile Tyr Trp Gln Lys His Asp Lys Val Val Leu Ser Val
30 35 40

Ile Ala Gly Lys Leu Lys Val Trp Pro Glu Tyr Lys Asn Arg Thr Leu
45 50 55 60

Tyr Asp Asn Thr Thr Tyr Ser Leu Ile Ile Leu Gly Leu Val Leu Ser
65 70 75

Asp Arg Gly Thr Tyr Ser Cys Val Val Gln Lys Lys Glu Arg Gly Thr
80 85 90

Tyr Glu Val Lys His Leu Ala Leu Val Lys Leu Ser Ile Lys Ala Asp
95 100 105

Phe Ser Thr Pro Asn Ile Thr Glu Ser Gly Asn Pro Ser Ala Asp Thr
110 115 120

Lys Arg Ile Thr Cys Phe Ala Ser Gly Gly Phe Pro Lys Pro Arg Phe
125 130 135 140

Ser Trp Leu Glu Asn Gly Arg Glu Leu Pro Gly Ile Asn Thr Thr Ile
145 150 155

Ser Gln Asp Pro Glu Ser Glu Leu Tyr Thr Ile Ser Ser Gln Leu Asp
160 165 170

Phe Asn Thr Thr Arg Asn His Thr Ile Lys Cys Leu Ile Lys Tyr Gly
175 180 185

Asp Ala His Val Ser Glu Asp Phe Thr Trp Glu Lys Pro Pro Glu Asp
190 195 200

Pro Pro Asp Ser Lys Asn Thr Leu Val Leu Phe Gly Ala Gly Phe Gly
205 210 215 220

Ala Val Ile Thr Val Val Val Ile Val Val Ile Ile Lys Cys Phe Cys
225 230 235

Lys His Arg Ser Cys Phe Arg Arg Asn Glu Ala Ser Arg Glu Thr Asn
240 245 250

Asn Ser Leu Thr Phe Gly Pro Glu Glu Ala Leu Ala Glu Gln Thr Val
255 260 265

Phe Leu
270

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 309 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION: 1..21

(D) OTHER INFORMATION: /label= signal_peptide

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 22..309

(D) OTHER INFORMATION: /note= "mature protein"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Asp Pro Arg Cys Thr Met Gly Leu Ala Ile Leu Ile Phe Val Thr
-20 -15 -10

Val Leu Leu Ile Ser Asp Ala Val Ser Val Glu Thr Gln Ala Tyr Phe
-5 1 5 10

Asn Gly Thr Ala Tyr Leu Pro Cys Pro Phe Thr Lys Ala Gln Asn Ile
15 20 25

Ser Leu Ser Glu Leu Val Val Phe Trp Gln Asp Gln Gln Lys Leu Val
30 35 40

Leu Tyr Glu His Tyr Leu Gly Thr Glu Lys Leu Asp Ser Val Asn Ala
45 50 55

Lys Tyr Leu Gly Arg Thr Ser Phe Asp Arg Asn Asn Trp Thr Leu Arg
60 65 70 75

Leu His Asn Val Gln Ile Lys Asp Met Gly Ser Tyr Asp Cys Phe Ile
80 85 90

Gln Lys Lys Pro Pro Thr Gly Ser Ile Ile Leu Gln Gln Thr Leu Thr
95 100 105

Glu Leu Ser Val Ile Ala Asn Phe Ser Glu Pro Glu Ile Lys Leu Ala
110 115 120

Gln Asn Val Thr Gly Asn Ser Gly Ile Asn Leu Thr Cys Thr Ser Lys
125 130 135

Gln Gly His Pro Lys Pro Lys Lys Met Tyr Phe Leu Ile Thr Asn Ser
140 145 150 155

Thr Asn Glu Tyr Gly Asp Asn Met Gln Ile Ser Gln Asp Asn Val Thr
160 165 170

Glu Leu Phe Ser Ile Ser Asn Ser Leu Ser Leu Ser Phe Pro Asp Gly
175 180 185

Val Trp His Met Thr Val Val Cys Val Leu Glu Thr Glu Ser Met Lys
190 195 200

Ile Ser Ser Lys Pro Leu Asn Phe Thr Gln Glu Phe Pro Ser Pro Gln
205 210 215

Thr Tyr Trp Lys Glu Ile Thr Ala Ser Val Thr Val Ala Leu Leu Leu
220 225 230 235

Val Met Leu Leu Ile Ile Val Cys His Lys Lys Pro Asn Gln Pro Ser
240 245 250

Arg Pro Ser Asn Thr Ala Ser Lys Leu Glu Arg Asp Ser Asn Ala Asp
255 260 265

Arg Glu Thr Ile Asn Leu Lys Glu Leu Glu Pro Gln Ile Ala Ser Ala
270 275 280

Lys Pro Asn Ala Glu
285

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 757 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

(A) NAME/KEY: exon

(B) LOCATION: 211..538

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 1

(D) OTHER INFORMATION: /note= "gap of approximately 3.4 kb between SEQ ID NO:1 and SEQ ID NO:11"

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 757

(D) OTHER INFORMATION: /note= "gap of approximately 2.0 kb between SEQ ID NO:11 and SEQ ID NO:12"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TGTTTCTACT CTGCTACTAT CTTAAGATGA ACAAACATAT GAACCTATAT ATTTCTCTGG 60

CATCACGTAG GAATGTAAAT AAATGTTTTC TTTTTTGCAT TTTCTCTTTC TTTTCTATAT 120

TTGACATTGA ATATACATTT TATTTATAAT CAGGAAAAAG TGTATGTGTG TTACAATCTT 180

TAAATATAAA TGTTCTTTTA TGCTTCCATA GTGCAAGTGG TGACCCAGGA TGAAAGAGAG 240

CAGCTGTACA CAACTGCTTC CTTAAAATGC TCTCTGCAAA ATGCCCAGGA AGCCCTCATT 300

GTGACATGGC AGAAAAAGAA AGCTGTAAGC CCAGAAAACA TGGTCACCTT CAGCGAGAAC 360

CATGGGGTGG TGATCCAGCC TGCCTATAAG GACAAGATAA ACATTACCCA GCTGGGACTC 420

CAAAACTCAA CCATCACCTT CTGGAATATC ACCCTGGAGG ATGAAGGGTG TTACATGTGT 480

CTCTTCAATA CCTTTGGTTT TGGGAAGATC TCAGGAACGG CCTGCCTCAC CGTCTATGGT 540

GAGAATCTCT GAGAATCATT GTCTGTGTCT GGAAATACTA TTTGCAAGAA TGTTTGGAAT 600

ATAGCCGTAG TGCCCAGTTT TTCAGGATTT TAACCACAGA AAGGGTCATG AGAAGATAGC 660

CTTTCTTGTC TACTATAGCT GTGTTTATGA TTATTTGGAG AGTTCATGGG GCTACACTGA 720

GTTCTTTGGC TGGAGCTATC TTTTCTCTGA GGGATCC 757

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 662 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

(A) NAME/KEY: exon

(B) LOCATION: 45..321

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 1

(D) OTHER INFORMATION: /note= "gap of approximately 2.0 kb between SEQ ID NO:11 and SEQ ID NO:12"

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 662

(D) OTHER INFORMATION: /note= "gap of approximately 1.3 kb between SEQ ID NO:12 and SEQ ID NO:19"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCAGGTGCTT AACTGATAAC AGATCATATT TATTTTTTGT CCCAGTACAG CCCATAGTAT 60

CCCTTCACTA CAAATTCTCT GAAGACCACC TAAATATCAC TTGCTCTGCC ACTGCCCGCC 120

CAGCCCCCAT GGTCTTCTGG AAGGTCCCTC GGTCAGGGAT TGAAAATAGT ACAGTGACTC 180

TGTCTCACCC AAATGGGACC ACGTCTGTTA CCAGCATCCT CCATATCAAA GACCCTAAGA 240

ATCAGGTGGG GAAGGAGGTG ATCTGCCAGG TGCTGCACCT GGGGACTGTG ACCGACTTTA 300

AGCAAACCGT CAACAAAGGT AAGAGAAAGT GAGCAAGGTG GCTGTGGTTG TGTCTGTGTG 360

CATGGACCTG GAAGGCAGTG AATGTCCTGC AGAGGTTTTC AGCCTCTTAG CATAATCTAT 420

TTGGAGAAAG AATGGGGCAA ATAAGGAAAA AACAAAACAA AACAAAAAAA TTGAAGAAAC 480

AAATAAAGCA AGTTTTACTT TCATAAATGT GGTTCATTGC CCACACACCA AATGCTGTTG 540

CTGAGATCCA TTCATTTATT TATTTCTTCC ACTCTACTAA CATGTAATTA GGTCTGTTCT 600

GGGCCAGACT TTGAGTTGAA CATGAAAAGT GCAGCAATGA GCAAGACTCA AGTCTCTAAG 660

CT 662

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CAGGCTGTAG GTGGACAGAT G 21

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GAAGTGGTGA CCCAGGATGA A 21

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GTATAATAAG ATGGAGATCA A 21

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TTGTTCATCC TGGGTCACCA CTTCCACTTG 30

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CTGGGTCACC ACTTCCACTT G 21

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ile Ser Gln Ala Val His Ala Ala His Ala Glu Ile Asn Glu Ala Gly
1 5 10 15

Arg

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 416 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

(A) NAME/KEY: exon

(B) LOCATION: 138..416

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 1

(D) OTHER INFORMATION: /note= "gap of approximately 1.3 kb between SEQ ID NO:12 and SEQ ID NO:19"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

AAATAATATG AAGTCATACG TATAAACCTA CATATGTATG TATTTAAGGA TAATTTTAAC 60

TAAAAGCTCA ACTCTTTTTT GCCTCAACAA TTTCCTCATG TGATGTCATT TTCCTTTTTC 120

TTTCTTCAAT ATCTATAGGC TATTGGTTTT CAGTTCCGCT ATTGCTAAGC ATTGTTTCCC 180

TGGTAATTCT TCTCGTCCTA ATCTCAATCT TACTGTACTG GAAACGTCAC CGGAATCAGG 240

ACCGAGGTGA GTTGTCACAG GGAGTTCAAA AAATGACATA AATTAAATTT GATTTTTAAT 300

GACAATTTGT GAGTCATTTG AAGATATAAA TAAGGGAATG GCAACAATGT GTTTTGTCTG 360

TTGTTTCCAA AATACAGAAA TGTTGATACT GTTTTAAAAT GCGTCGGGGC ATTTTC 416

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

AGGATCCTTG TCCAGACTCT GCTT 24

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ATAGGATCCG CCGCCACCAT GCCCTTCTCT CATCTGTCT 39

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

ATGGATCCCC TTTGTTGACG GTTTGCTT 28

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Gly Asp Pro Arg

1

CLAIMS

1. A method for modulating a T-cell mediated immune response in a subject diagnosed as having a condition that is treatable by modulation of the subject's T-cell mediated immune response, the method comprising:

(a) administering to the subject a therapeutically effective amount of an OX-2 therapeutic agent to modulate the T-cell mediated immune response and thereby treat the condition.

2. The method of claim 1, wherein modulating comprises up regulating a T-cell mediated immune response to an antigen.

3. The method of claim 1, wherein modulating comprises down regulating a T-cell mediated immune response to an antigen.

4. The method of claim 2, wherein the condition that is treatable by up regulation of the T-cell mediated immune response is selected from the group consisting of an immunosuppressive disorder, a neoplastic condition and an infection.

5. The method of claim 3, wherein the condition that is treatable by down regulation of the T-cell mediated immune response is selected from the group consisting of an autoimmune disease, graft vs. host disease and transplant rejection.

6. The method of claim 1, wherein the OX-2 therapeutic agent is selected from the group consisting of OX-2, an OX-2 agonist, and an OX-2 antagonist.

7. The method of claim 6, wherein the OX-2 agonist comprises a soluble form of OX-2 selected from the group consisting of a chimeric peptide containing the extracellular domain of OX-2, a chimeric peptide containing the IgV-like domain of OX-2 and a chimeric peptide containing the IgV-like domain of OX-2.

8. The method of claim 7, wherein the chimeric peptide comprises a chimeric immunoglobulin polypeptide comprising:

(a) at least one amino acid sequence selected from the group consisting of the extracellular domain of

OX-2, the IgV-like domain of OX-2 and the IgC-like domain of OX-2, the amino acid sequence being covalently coupled at its C-terminus to

(b) an N-terminus of an immunoglobulin constant region amino acid sequence.

9. The method of claim 6, wherein the OX-2 therapeutic agent comprises an OX-2 antagonist selected from the group consisting of an antibody to OX-2, an antisense nucleic acid that hybridizes under stringent conditions to a nucleic acid encoding OX-2; and an OX-2 agonist that has been modified to prevent crosslinking to an OX-2 receptor.

10. A method for diagnosing a subject having a condition that is treatable by administration of an OX-2 therapeutic agent, the method comprising:

(a) contacting a biological sample of the subject with an OX-2 diagnostic agent to detect the presence of OX-2 in the biological sample.

11. The method of claim 10, wherein the biological sample comprises a lymphatic tissue.

12. The method of claim 10, wherein the biological sample comprises a cell-containing sample, wherein the cells are selected from the group consisting of T-cells, thymocytes, neurons, endothelial cells, B cells and dendritic cells.

13. A method for enhancing the effectiveness of a vaccine containing an antigen to a subject, the method comprising:

coadministering to the subject a therapeutically effective dose of an OX-2 therapeutic agent to up regulate the T-cell mediated immune response of the subject to the antigen.

14. The method of claim 13, wherein the vaccine comprises a vaccinia virus that expresses the antigen and an OX-2 therapeutic agent selected from the group consisting of OX-2 and an OX-2 agonist.

15. A chimeric immunoglobulin polypeptide comprising:

(a) at least one amino acid sequence selected from the group consisting of the extracellular domain of OX-2, the IgV-like domain of OX-2 and the IgC-like domain of OX-2, the amino acid sequence being covalently coupled at its C-terminus to

(b) an N-terminus of an immunoglobulin constant region amino acid sequence.

16. The polypeptide of claim 15, wherein the polypeptide is a polypeptide having at least one characteristic selected from the group consisting of (a) the peptide does not bind to the B7 receptors CTLA4 and CD28; (b) the polypeptide costimulates CD4-- T cells; and (c) the polypeptide costimulates thymocytes in an antigen dependent context.

17. A composition comprising an antibody that specifically binds to an OX-2 protein having a T cell costimulatory activity and inhibits the T cell costimulatory activity of the OX-2 protein to which it is bound.

18. A diagnostic kit for detecting the presence of an OX-2 nucleic acid or an OX-2 protein in a biological sample, the kit comprising an OX-2 diagnostic agent, and instructions for using the OX-2 diagnostic agent to detect the presence of the OX-2 nucleic acid or the OX-2 protein in a biological sample.

19. A vaccine comprising:

a therapeutically effective amount of an antigen to elicit an immune response in a subject and a therapeutially effective amount of an OX-2 therapeutic agent selected from the group consisting of OX-2 and an OX-2 agonist to enhance the immune response in the subject to the antigen.

	αrOX-2	αIAd	αB7-1	mCTLA4Ig	mCD28Ig
CHO	—	—	—	—	—
CHO-IAd	—	+++	—	—	—
CHO-IAd-B7-1	—	+++	+++	+++	+
CHO-IAd-rOX-2	+++	+++	—	—	—

FIG. 1

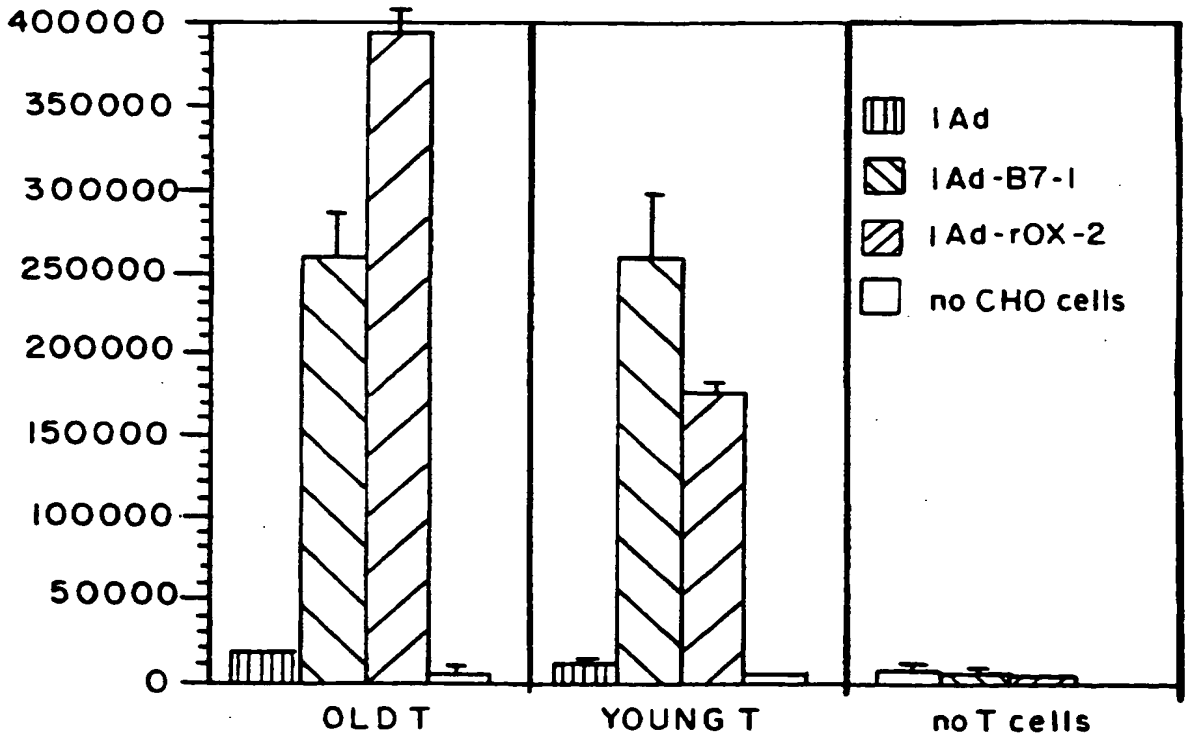

FIG. 2A

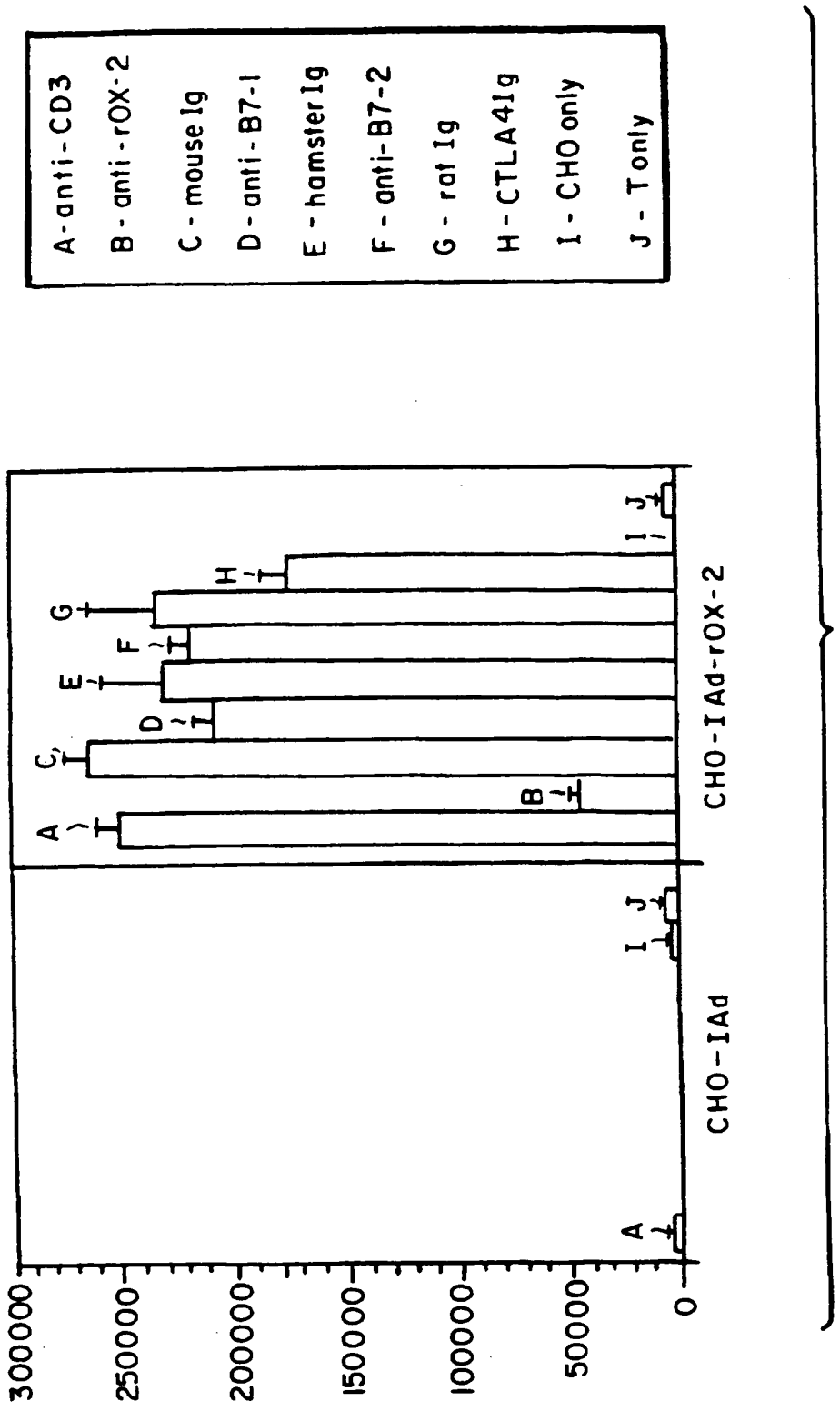
A - anti - CD3
B - anti - rOX-2
C - mouse Ig
D - anti - B7-1
E - hamster Ig
F - anti - B7-2
G - rat Ig
H - CTLA4Ig
I - CHO only
J - T only
300000
250000
200000
150000
100000
50000
0
CHO-IAd
CHO-IAd-rOX-2

FIG.2B

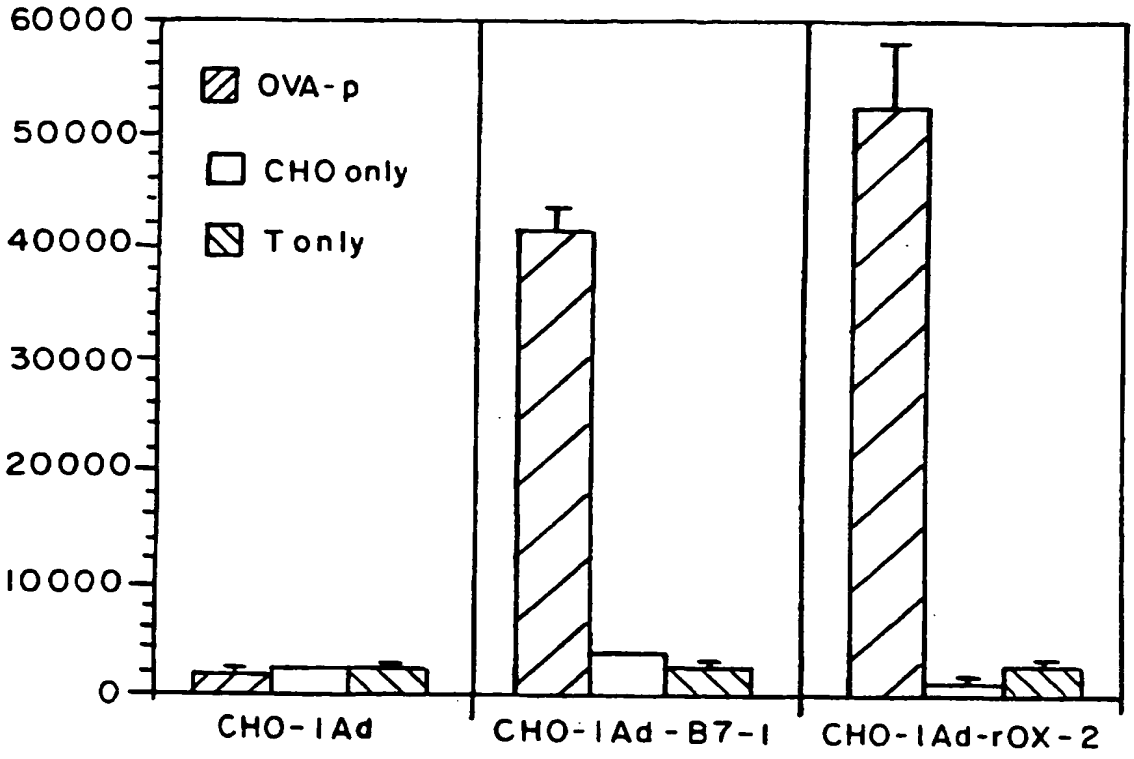

FIG. 2C

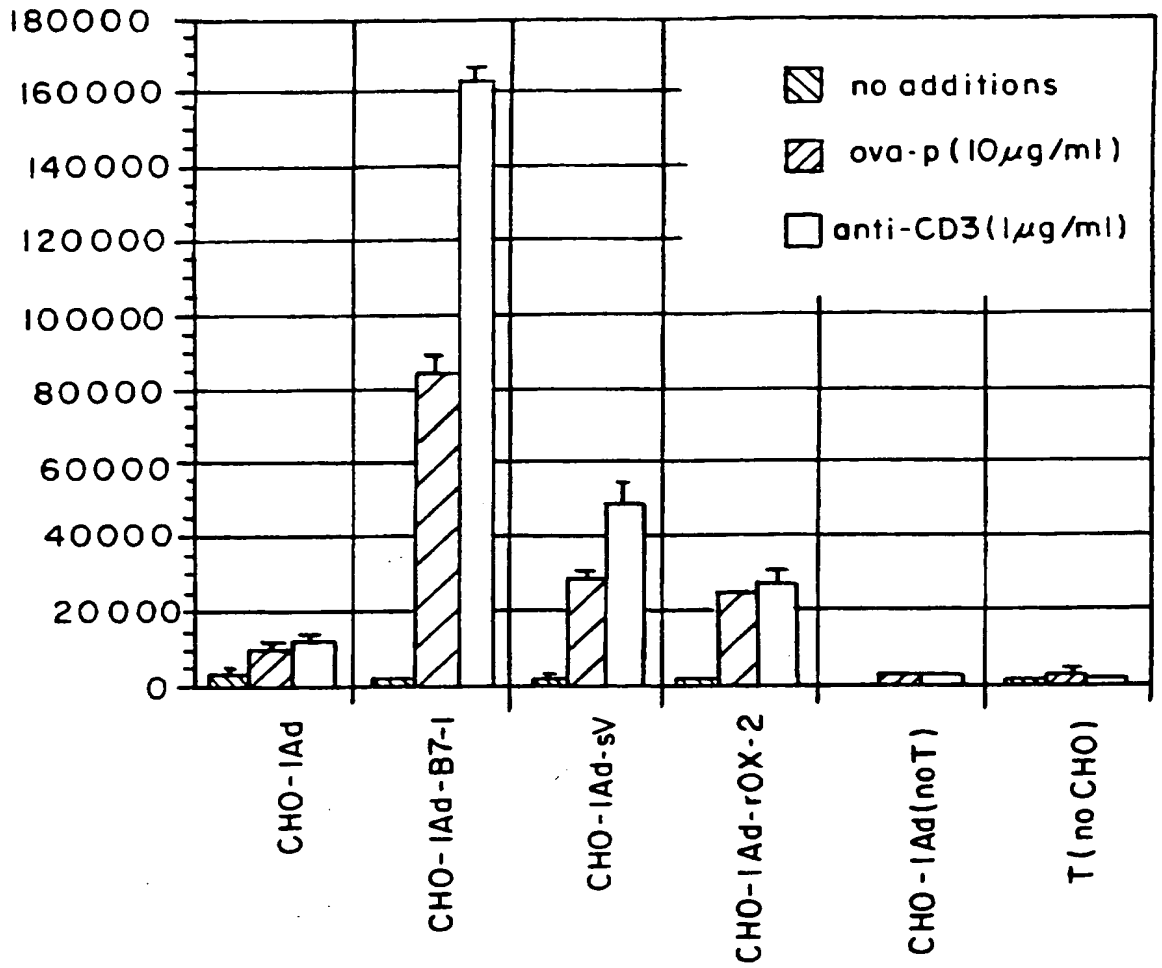
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
no additions
ova-p (10μg/ml)
anti-CD3(1μg/ml)
CHO-1Ad
CHO-1Ad-B7-1
CHO-1Ad-sV
CHO-1Ad-rOX-2
CHO-1Ad(noT)
T(no CHO)

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/19189

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 39/00, 39/395; C07H 21/02, 21/04; C12Q 1/00; G01N 33/53

US CL : 424/130.1, 133.1, 178.1, 184.1; 435/4, 7.1; 536/23.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1, 133.1, 178.1, 184.1; 435/4, 7.1; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BARCLAY et al. Neuronal/Lymphoid Membrane Glycoprotein MRC OX-2 is a Member of the Immunoglobulin Superfamily with a Light-Chain-Like Structure. Biochem. Soc. Symp. 1985, Vol. 51, pages 149-157.	1-9, 13-16, 19
A	MCCAUGHAN et al. Characterization of the Human Homolog of the Rat MRC OX-2 Membrane Glycoprotein. Immunogenetics. 1987, Vol. 25, pages 329-335.	1-9, 13-16, 19
Y	PATERSON et al. Antigens of Activated Rat T Lymphocytes Including A Molecule of 50,000 Mr Detected Only on CD4 Positive T Blasts. Molecular Immunology. 1987, Vol. 24, No. 12, pages 1281-1290, especially pages 1281 and 1288.	1-4, 6

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
26 FEBRUARY 1997

Date of mailing of the international search report
26 MAR 1997

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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No
PCT/US96/19189

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	MJAALAND et al. Modulation of immune responses with monoclonal antibodies I. Effects on regional lymph node morphology and on anti-hapten responses to haptenized monoclonal antibodies. Eur. J. Immunol. 1990, Vol. 20, pages 1457-1461, especially Abstract and pages 1459-1461.	13, 19 --------- 1, 2, 4, 6, 14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/19189

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

BIOSIS, MEDLINE, EMBASE, WPIDS, USPATFULL, REGISTRY, CAPLUS, CA, LIFESCI, DRUGU, SCISEARCH, CABA, JICST-EPLUS, PHIN, CANCERLIT, TOXLINE, CONFSCI, DISSABS, TOXLIT, CEN, CJACS, NTIS
search terms: ox-2, immun?, modulat?, T cell, T cell immune response, endothelial cell?, b cell?, dendrit? cell?, costimulat? agonist, antagonist, antibod?, antisense, Borriello, Sharpe, ox 2